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• ***Dr. Kanchanjunga Prasai***

DNA DAMAGE CONTROLLING SYSTEMS IN MITOCHONDRIA Dr. Kanchanjunga Prasai

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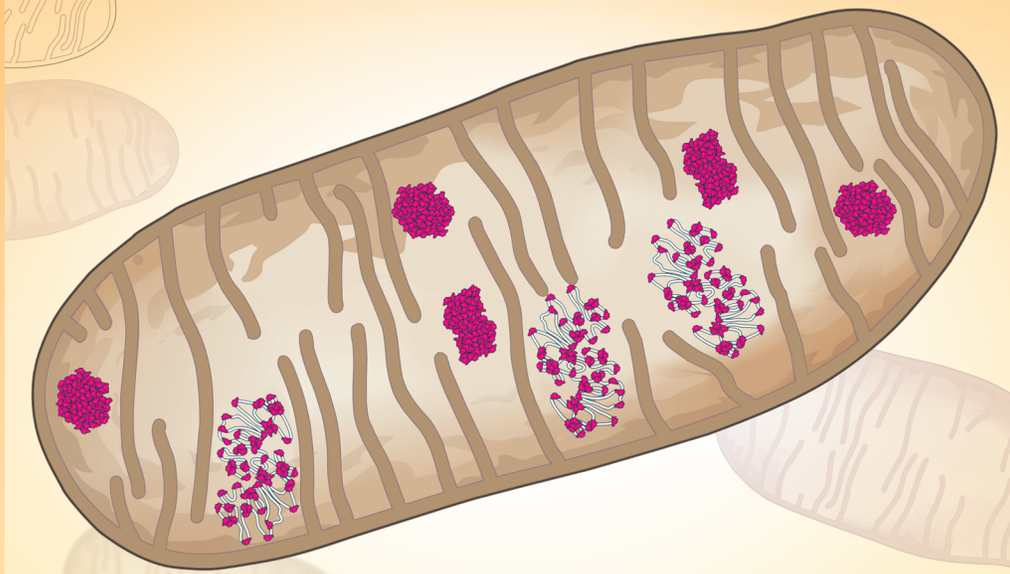

Dr. Kanchanjunga Prasai

DNA DAMAGE CONTROLLING SYSTEMS IN MITOCHONDRIA

Nai Prakashan

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Kanchanjunga Prasai, M.Sc., Ph.D.

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DNA DAMAGE CONTROLLING SYSTEMS IN MITOCHONDRIA
by Kanchanjunga Prasai, M.Sc., Ph.D.

To my grandmother

Bhagiratha Prasai

(1919-2012)

whose eternal blessing has enabled me to be the person I am today.

ABOUT THE AUTHOR

Dr. Kanchanjunga Prasai was born and raised in Kathmandu, Nepal. He received his I.Sc. (equivalent to a high school diploma) from Kathmandu University, where he developed an interest in Biochemistry. He obtained his B.Sc. in Biochemistry from the University of Madras, India, in 2005 and his M.Sc. in Biochemistry from Hemvati Nandan Bahuguna Garhwal University, India, in 2007. In 2008, he joined the faculty of the Department of Biochemistry at the Institute of Medicine, Kathmandu, as a Teaching Assistant, and was promoted to Assistant Professor in 2010. He remained at the institute until 2011, and during his three-year stint, he taught biochemistry to bachelor's level medical, nursing, and allied health students.

In the fall of 2011, Dr. Prasai received a scholarship to pursue a doctorate in the Department of Molecular and Cellular Physiology at Louisiana State University Health Sciences Center-Shreveport (LSUHSC-Shreveport), USA, where he got trained under the supervision of Prof. Dr. Lynn Harrison. His research interest focused on mitochondrial DNA damage, repair, and replication. One of his responsibilities as a Ph.D. student was to teach endocrine physiology to master's level allied health students. During his Ph.D. program, he received the Carroll Feist Predoctoral Fellowship Award (2013-2016) from the Feist-Weiller Cancer Center, LSUHSC-Shreveport, to fund his Ph.D.

student stipend. He has also received several other awards for his research on mitochondrial DNA damage, repair, and replication. These include the Young Investigator Award (2014) to attend the 13th International Workshop on Radiation Damage to DNA held at the Massachusetts Institute of Technology, USA, and the Jason A. Cardelli Award for Excellence in Cancer Research (2015) at the Ray A. Barlow Scientific Symposium held in the premises of LSUHSC-Shreveport.

After receiving his Ph.D. in 2017, Dr. Prasai joined the lab of Prof. Dr. Rona Scott in the Department of Microbiology and Immunology at LSUHSC-Shreveport, where he remained until 2019. During his spell in the Scott Lab as a postdoctoral scientist, he studied the role of lymphoid enhancer-binding factor-1 in the Epstein-Barr virus life cycle. He has a number of publications to his name in peer-reviewed international journals. Even though his field of interest is mitochondrial biology, he has contributed as a co-author to areas as diverse as virology and microgravity. Dr. Prasai aspires to a career in academia and enjoys writing, teaching, and conducting research.

PUBLISHER'S NOTE

Nai Prakashan (Nai Academy), ever since its establishment on January 29, 1996, has been deeply involved in the world of Nepali Language – concentrating on the idea of 'universal brotherhood'. Founded with the aim of uplifting 'Nepali Literature', it has always upheld norms and values of tradition and culture, and will always continue to do so. Recognition of talents in the country and abroad is one of the main objectives of this literary organization.

Nai Prakashan has been engaged in identifying and appreciating talents in the field of Nepali art and culture, language and literature, social services, science and technology, school of thoughts, games and sports, and alike. It has also been actively engaged in conducting various cultural programs from time to time as well as in the publication of different series of books.

Nai Prakashan, ever adhering to pure Nepali values, norms, and accepted standards, is well on course to give exposure to every talent's constructive skills in a nice and pleasant manner. Indeed, after being born and cherishing a life of purpose, it is the solemn duty of each and everyone to dedicate oneself to singing in praise of one's motherland. In this context, this organization is proudly inspired by the belief that service in the literary field is also an important aspect of service to the whole nation.

This book *DNA Damage Controlling Systems in Mitochondria* has been published in line with various plans, programs, and objectives of Nai Prakashan.

Dr. Kanchanjunga Prasai is a scientist by profession with a doctorate in mitochondrial DNA biology from an American University. Also the Treasurer of Nai Prakashan, Dr. Prasai has significantly contributed to the collection of books published by this organization. Apart from authoring *DNA Damage Controlling Systems in Mitochondria*, he is the editor of *Shree Swasthani Bratakatha* (a theological book) and has translated *My Mother Bhagiratha Prasai* (a biography) from Nepali to English.

Mitochondrial DNA damage occurs naturally within a cell. In this book, the author has portrayed different mitochondrial DNA damage controlling systems, which include mitochondrial DNA repair, mitochondrial DNA degradation, sanitation of premutagenic free nucleotides, and translesion synthesis.

Nai Prakashan thanks the author Dr. Kanchanjunga Prasai for preparing *DNA Damage Controlling Systems in Mitochondria* and values the glorious occasion of publishing this book as a golden opportunity.

• Nai Prakashan

PREFACE

My curiosity for mitochondria arose about 10 years ago when I was rotating as a Ph.D. student in the Harrison Lab in the Department of Molecular and Cellular Physiology at Louisiana State University Health Sciences Center-Shreveport, USA. Prof. Dr. Lynn Harrison, a nuclear DNA repair expert, was also interested in mitochondrial DNA (mtDNA) repair. After joining her lab as a full-time Ph.D. student, my fascination for the "mighty mitochondria" further increased. As a Ph.D. student, I was interested in mtDNA damage, repair, and replication, and during that period, I was able to produce three first-author articles in peer-reviewed international journals on mitochondrial biology. After earning a Ph.D., I joined the lab of Prof. Dr. Rona Scott as a postdoctoral scientist in the Department of Microbiology and Immunology at the same institution. Even though Prof. Dr. Scott's field of interest was virology, she constantly motivated me to continue studying mitochondrial biology while I was in her lab.

MtDNA biology is a rapidly evolving field with major advancements witnessed in the last few decades. MtDNA damage and mutations can lead to mitochondrial dysfunction and associated diseases including type 2 diabetes, cardiovascular diseases, and cancer. Fortunately, eukaryotic cells have a diverse suite of DNA damage controlling systems in mitochondria that act in concert to maintain an error-free mitochondrial genome. One of the main

characteristics of this book is that it touches different domains of mtDNA damage controlling systems, from mtDNA repair to sanitation of premutagenic free nucleotides. However, few areas can be seen as relatively neglected or missing. For example, DNA polymerase theta is fairly little mentioned, which is due to the scarcity of information about the protein's potential involvement in translesion synthesis in mitochondria. As the main mtDNA damage controlling system, this book focuses on mtDNA repair, especially on base excision repair and double-strand break repair. All known human DNA glycosylases, enzymes that initiate the base excision repair pathway, have been presented and those which localize to mitochondria have also been mentioned. To make it easier for the reader to understand the pathway, a schematic representation of the base excision repair has been depicted. This book also contains several other figures and tables that will, hopefully, be beneficial to the reader by helping him/her visually interpret the text.

MtDNA degradation is another important topic that this book emphasizes. Recent advancements in biochemistry, molecular biology, and genetics have made it possible to identify different proteins participating in mtDNA degradation, and this book attempts to present the most up-to-date information on our current understanding of this phenomenon. Towards the end of the book, the significance of translesion synthesis and enzymes potentially participating in this novel process are discussed and examined. What one may find as an interesting aspect of this book is that it discusses multiple mitochondrial polymerases, besides DNA polymerase gamma, which is in contrast to the initial belief that DNA polymerase gamma is the only DNA polymerase acting in mitochondria. Collectively, after decades of study, we now know that mitochondria, like the nucleus, do harbor specific mechanisms to maintain the integrity of its small and polyploid genome. As mtDNA damage can lead to organ dysfunction and diseases, an overall

understanding of mtDNA damage controlling systems can provide insights into the development of therapeutics for different diseases.

While I was a Ph.D. student, I perceived the lack of a book or an article providing basic information on mitochondria, mtDNA, mtDNA damage, and different systems that tackle damage inflicted to the mitochondrial genome. This book, therefore, has been prepared with an intention to fill that void and to provide the aforementioned information to the reader. Also, being a citizen of a developing country like Nepal, I feel most Nepalese students and scientists are oblivious of the significance of mtDNA biology. Publishing this book is an endeavor to introduce the importance of mtDNA damage controlling systems to Nepalese students and scientists, and to ignite their interest in these fascinating fields. I look forward to witnessing the positive impact that this book would make on Nepalese academia. It is also hoped that this book will influence Nepalese students and scientists to ask fundamental questions on mtDNA biology and DNA damage controlling systems in mitochondria. This book is expected to serve as a reference book and is by no means prepared as a textbook, with a glossary and review questions and exercises. A bibliography has been compiled to provide the interested reader a start in exploring previous researches. It is likely that multiple relevant studies are being published as this book is being prepared; however, they should not make the basic principles and insights present in this book outdated.

In the end, I would like to express my deepest gratitude to several individuals who have encouraged and helped me in many ways. Firstly, I most sincerely acknowledge my Ph.D. mentor Prof. Dr. Lynn Harrison for arousing my interest in mitochondrial biology. I extend my heartfelt appreciation to my postdoctoral mentor Prof. Dr. Rona Scott for motivating me to broaden my knowledge in the same field. I thank Mr. Krishna Gopal Shrestha

for taking the time to design the cover of this book. Last but not least, I am indebted, as ever, to my parents Mr. Narendra Raj Prasai and Mrs. Indira Prasai for constantly encouraging and supporting me to prepare the manuscript of this book.

• **Kanchanjunga Prasai, M.Sc., Ph.D.**

CONTENTS

DNA DAMAGE CONTROLLING SYSTEMS IN MITOCHONDRIA

1
SNAPSHOT

Mitochondria are essential double membrane organelles within a cell that harbor their own genetic material, the mitochondrial DNA (mtDNA), which encodes indispensable protein components of the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS). Apart from generating the majority of the cell's adenosine triphosphate (ATP) that drives a multitude of reactions necessary for life, the OXPHOS machinery is also the primary source of reactive oxygen species (ROS). Since mtDNA is located in close proximity to the ROS generating factory and is also not protected as complex nucleosomes like the nuclear counterpart, mtDNA is under constant threat not only from ROS but also from environmental factors like ionizing radiation. These endogenous and exogenous agents have the potential to damage mtDNA molecules, which can result in the loss of mtDNA integrity. As mtDNA encodes protein components of the ETC and OXPHOS, failure to tackle mtDNA lesions can lead to disruption of the ETC and enhanced ROS production, which can, in turn, result in energy depletion, cell death, organ dysfunction, and ultimately disease. Therefore, maintaining the stability of mtDNA is critical not only for mitochondrial and cellular function but also for organismal fitness. This book focuses on our current knowledge of different mtDNA repair mechanisms that help preserve the integrity of

the mitochondrial genome. Our current comprehension of the degradation of damaged mtDNA molecules and molecular players involved in the process are discussed. Finally, the roles of sanitation of premutagenic free nucleotides in mitigating mtDNA damage and translesion synthesis as an important strategy in tolerating damage to the mitochondrial genome are also summarized.

2
INTRODUCTION

Around 2 billion years ago, when the Earth's atmosphere started to become abundant in oxygen, a primeval eukaryotic cell incapable of using oxygen engulfed aerobic bacteria. Escaping digestion, these bacteria established an endosymbiotic relationship with the engulfing cell: the bacteria obtained shelter and nourishment from the host and in return provided energy to the host to meet its bioenergetic demands. Eventually, in 1898 the engulfed bacteria were named mitochondria by a German physician Carl Benda; the word derived from the Greek "mitos" (thread) and "chondros" (granule), describing the appearance of these entities during spermatogenesis. The endosymbiotic theory of the origin of mitochondria is now universally acknowledged; the theory is supported not only by the fact that these organelles harbor their own genetic material but also by the observation that they contain their own protein-synthesizing machinery committed to translating mitochondrial genes. In addition, the close homology of bacterial and mitochondrial respiratory chain complexes and other membrane proteins along with the ability of mitochondria to divide further provide evidence in support of the theory. Endosymbiosis is now considered a fundamental force that steered eukaryotic evolution. Indeed, the acquisition of mitochondria by a proto-eukaryote is believed to have supplied energy essential for the

enlargement of the nuclear genome, and this, with time, enabled the evolution of complex organisms [1].

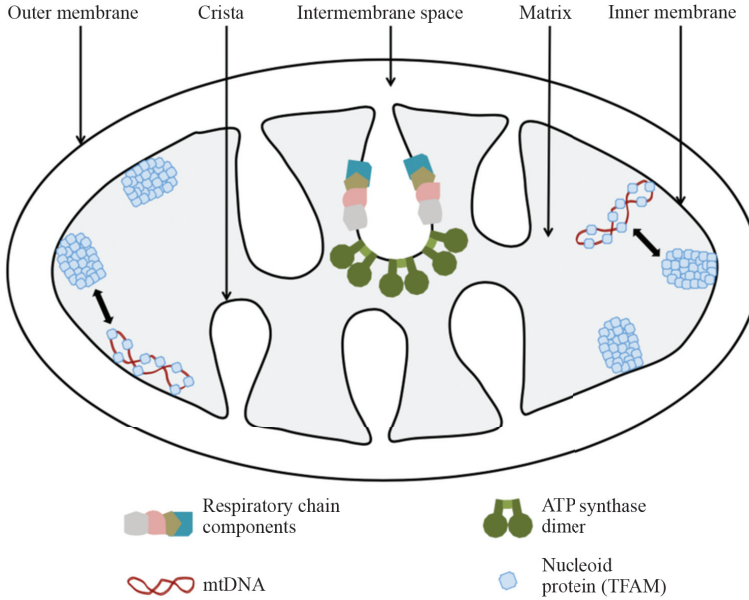

Figure 1 Structure of a mitochondrion. A highly specialized double membrane encloses the mitochondrion. The respiratory chain complexes and ATP synthase in mitochondria are preferentially located in the cristae membrane. MtDNA molecules are organized in nucleoprotein particles called nucleoids. TFAM (transcription factor A, mitochondrial) is the most prominent component of nucleoid-associated protein in mammalian cells. Different degrees of nucleoid compaction are observed at physiological TFAM/mtDNA ratios, so poorly compacted nucleoids can therefore be noticed next to fully compacted ones. At high TFAM/mtDNA concentrations, TFAM can fully coat and compact mtDNA, which is then blocked for replication and transcription. This figure is adapted from [1] and [2].

Mitochondria are present in almost all eukaryotic cells [3]. Those eukaryotes that do not contain mitochondria once possessed the organelles and eventually lost them [4-6]. In regard to humans, they are found in all nucleated cells. Each nucleated human cell generally contains several hundreds of cytoplasmic mitochondria,

a feature that depends on the tissue energy demands [7]. They have a characteristic double lipid bilayer membrane structure and are compartmentalized (Figure 1). The lipid and protein compositions differ between the outer and inner membranes of mitochondria. The inner membrane contains many finger-like projections called cristae (singular: crista) and the membranes of such structures are called cristae membranes (CM). The respiratory chain complexes and the F_1F_0-ATP synthase that produce ATP via OXPHOS are preferentially localized in the CM. Indeed, rows of F_1F_0-ATP synthase dimers are present along the edges and tips of cristae and this arrangement generates a high membrane curvature, providing cristae their characteristic shape. Mitochondria also have functionally distinct intermembrane and matrix spaces. Apart from generating the majority of a cell's ATP, mitochondria also perform a variety of other vital functions including biogenesis and assembly of iron-sulfur proteins, biosynthesis of heme and steroid hormones, maintenance of calcium homeostasis, activation of apoptosis, just to name a few [1]. They accomplish these functions through the synergistic activities of about 1500 proteins present in the mitochondrial compartment [7, 8]. These unique organelles also harbor their own genetic material, the mtDNA [8], which occurs in multiple copies per cell, a characteristic distinct from the nuclear counterpart. MtDNA is packaged by association with specific proteins in compact DNA-protein complexes, called nucleoids, which are closely associated with the inner membrane. In general, the mtDNA contains genes for a small number of mitochondrial proteins together with RNA components of the mitochondrial protein-synthesizing machinery. In addition to a small fraction of proteins (13 total in humans [9]) encoded by the mitochondrial genome, mitochondria import more than 99% of other proteins that are encoded by the nuclear genes and synthesized in the cytoplasm [7]. Thus, mitochondria are under dual genome control and the coordination of products of both

the nuclear and mitochondrial genomes are necessary for ATP production and cellular function [7, 10]. The location of mtDNA and, possibly, its structural organization contribute to a higher DNA damage accumulation when compared to its nuclear counterpart [11]. As mtDNA encodes essential components of the ETC and OXPHOS, defective mtDNA results in the insufficient synthesis of the mtDNA-encoded ETC complex subunits, and these events can further impair OXPHOS causing energy deficiency, eventually resulting in organ dysfunction and disease [7]. Because the integrity of the mitochondrial genome is indispensable for mitochondrial and cellular function as well as organismal fitness, several systems act upon to preserve that, which include mtDNA repair, degradation of the damaged genome, sanitation of premutagenic free nucleotides, and translesion synthesis. This book portrays mtDNA structure and events that lead to damage to mtDNA. I then focus on different repair mechanisms that help maintain the integrity of the mitochondrial genome. Finally, other systems that assist in controlling mtDNA damage and enzymes that potentially participate in damage tolerance are also discussed.

3

MITOCHONDRIAL DNA STRUCTURE, TOPOLOGY, AND ORGANIZATION

MtDNA is a fossil molecule and its residence in the mitochondrial matrix substantiates the idea that endosymbiosis did occur [12]. It is assumed that the mitochondrial genome, at the time of the endosymbiotic event, was as large as those of the modern α-proteobacteria [13], the closest contemporary relative of the ancestral eubacterial symbiont [14]. During evolution, however, the majority of the genetic material of the α-proteobacterium progenitor was either lost or transferred to the nuclear genome, and this phenomenon is believed to be a part of the process for acquiring new functions by mitochondria [15, 16]. Modern-day mitochondria, thus, harbor remnants of the bacterial genome, which, in general, contains genes for multiple mitochondrial proteins together with components of the protein-synthesizing machinery [13]. The ~16.6 kilobase pairs (kb) human mtDNA, for example, encodes 13 protein components of the OXPHOS system along with 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) [9, 13, 15]. Different eukaryotic species, however, display striking heterogeneity in size, physical form, and coding capacity of their mtDNA. The human malarial parasite *Plasmodium falciparum*, for instance,

possesses the smallest known mitochondrial genome of ~6 kb that harbors 2 rRNA genes and 3 protein-encoding genes [17, 18]. Another interesting feature of this organism is the linearity of its mtDNA, a topology found in a wide variety of organisms including *Saccharomyces cerevisiae* (fungi), *Euglena gracilis* (single-celled flagellate), and *Chenopodium album* (plant) [19]. In contrast to the mtDNA of *P. falciparum*, a flowering plant *Silene conica* contains the largest known mitochondrial genome of ~11.3 megabase pairs, which exceeds the size of most bacterial genomes and even some nuclear genomes; nonetheless, it contains only 30 genes − 25 for proteins, 3 for rRNAs and 2 for tRNAs [20]. Intriguingly, mtDNA in *S. conica* has fragmented into dozens of 'chromosomes' (multichromosomal structure), with the vast majority (98.2%) of sequence content assembled into 128 circular-mapping chromosome units that range from 44 to 163 kb in size [20]. Even though mitochondrial genome is typically perceived to comprise of a single 'chromosome', cases of multiple mapping mtDNA molecules have been reported in a variety of species. For example, the mitochondrial genome in a fungus *Spizellomyces punctatus* [14, 21, 22] consists of three distinct circular mapping molecules, whereas a protist *Amoebidium parasiticum* has several hundred distinct types of linear mtDNA molecules [14, 23].

With respect to the coding capacity of mtDNA, the freshwater protozoan *Reclinomonas americana* is a remarkable example, which contains the largest compendium of mitochondrial genes identified so far in any eukaryotic species. Harboring 97 genes, its ~69 kb circular mitochondrial genome encodes not only for components of the OXPHOS system and protein translational machinery but also for those involved in protein import, protein maturation, and mtDNA transcription [24]. A number of these genes are either completely absent or very rare in mtDNA from other species but exist in bacterial genomes [25]. Its mtDNA also exhibits

other striking features of a bacterial genome, including operon-like gene clusters and putative Shine-Dalgarno motifs upstream of protein-coding genes. Because of its marked resemblance to a typical bacterial genome, *R. Americana* mtDNA has been referred to as "a eubacterial genome in miniature" [17, 24].

3.1 Budding Yeast Mitochondrial DNA

In 1963, a groundbreaking experiment using chick embryos led to the first clear identification of mtDNA [26], and a year later, budding yeast mitochondria were also revealed to possess their own genome [27]. Soon after, mtDNAs from some vertebrate species were isolated, and these extranuclear DNA molecules were shown to exist in a closed circular duplex form [19, 28, 29]. Reinforcing the idea that mitochondria had originated from bacteria, which, in general, were known to possess circular genomes, the scientific community worldwide assumed that yeast mtDNA also had circular topology [19]. This false belief persisted until 1991 when pulsed-field gel technology, for the first time, demonstrated that yeast mitochondrial genome predominantly existed in linear form [30]. These linear molecules are of variable sizes and occur as head-to-tail multimers (concatemers) of several genomic units. Moreover, yeast mitochondria also possess a small number of circular monomeric mtDNA molecules, which are believed to serve as templates for replication by the rolling-circle mechanism to produce polydisperse linear tandem arrays of the genome [19, 30-32]. Interestingly, concatemers constitute the major species of mtDNA in mother cells and non-dividing cells, whereas monomeric circles predominate in growing buds [33]. It is not clear, however, if circles are the functional units and the linear species act as replication intermediates or if circular forms are unimportant for mitochondrial function [34].

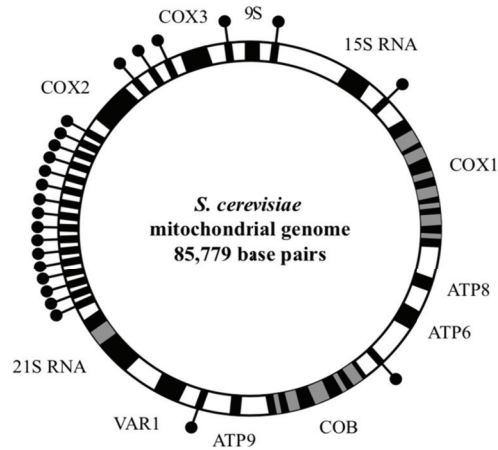

Figure 2 The map of *S. cerevisiae* mitochondrial genome. The circular monomeric mtDNA map reveals the locations of all genes, which are represented as black boxes. Intronic regions within *COX1*, *COB*, and 21S rRNA genes are indicated by grey boxes. Locations of tRNA genes are represented as sticks with filled circles. Even though the yeast mitochondrial genome is represented in a circular form, the majority of the yeast mtDNA molecules exist as linear concatemers. This figure is adapted from [45].

The budding yeast mitochondrial genome accounts for ~15% of total cellular DNA, which is equivalent to ~50 monomeric copies of the ~75−85 kb genome per haploid cell [19, 35]. The 85,779 base pairs (bp) monomer [35] measures over 25 μm [36] and is characterized by low gene density with only 8 protein-encoding genes, namely for cytochrome *c* oxidase subunits 1, 2, and 3 (*COX1*, *COX2*, and *COX3* genes, respectively), ATP synthase subunits 6, 8, and 9 (*ATP6*, *ATP8*, and *ATP9* genes, respectively), apocytochrome *b* (*COB* gene), and a ribosomal protein Var1 (*VAR1* gene) [37, 38] (Figure 2). In addition, the mitochondrial genome contains genes for 2 rRNAs (15S and 21S), 24 tRNAs, the 9S RNA component of RNase P (involved in the processing of mitochondrial tRNA precursors), and seven to eight replication-origin like elements [38]. Furthermore, the genome sequence contains several dispensable open reading frames and up to 13

introns; the latter are distributed among three different genes, viz., *COX1*, *COB*, and 21S rRNA. Several of these introns encode open reading frames that are translated to produce maturases, reverse transcriptases, or site-specific endonucleases, and these dispensable proteins are involved in events like intron splicing and intron mobility in mitochondria [37, 39-44].

Characterized by low guanine-cytosine (GC) content (17−18%), yeast mtDNA contains a substantial amount of intergenic regions, which accounts for 62% of the genome [35, 46]. Interestingly, the adenine-thymine (AT) and GC base pairs are highly clustered in yeast mtDNA. The AT-rich sequences, which range from 150-1500 bp, comprise ~50% of the entire mtDNA sequence. On the other hand, the GC-rich sequences, commonly referred to as GC-clusters, are ~50-80 bp long and constitute ~2-3% of the total mtDNA. Most of these GC clusters are dispersed throughout the mtDNA, but are particularly enriched in the intergenic regions, and have been recognized as the preferential site of recombination in yeast mtDNA [47].

3.2 Budding Yeast Mitochondrial Nucleoid

Cellular DNA molecules do not occur as 'naked' but are rather shielded by a variety of proteins in intricate DNA-protein complexes. MtDNA molecules are no exception to this rule, as they are organized in nucleoprotein particles called nucleoids, a term used analogously to DNA-organizing structures in bacteria. Each eukaryotic organism appears to have a distinct set of nucleoid-associated proteins that condense, protect, and regulate the activities of mtDNA molecules. In most organisms, nucleoids contain 25 or more proteins, most of which are involved in nucleoid organization, mtDNA transactions (replication, transcription, repair, and recombination), protein quality control, and metabolism [36, 48]. The fact that proteins involved in mtDNA transactions occur in a nucleoid structure substantiates the notion that mitochondrial

nucleoids (mt-nucleoids) are units of mtDNA inheritance that are transmitted to daughter cells with high fidelity [49].

In *S. cerevisiae*, nucleoids appear to be evenly spaced along the mitochondrial reticulum, tethered to the inner membrane [32]. Each yeast cell can contain ~10-40 nucleoids [32], with each nucleoid comprising of ~35 proteins [50] that can shelter up to 10 mtDNA molecules [10]. Moreover, each nucleoprotein particle appears somewhat spherical with a diameter of ~400 nm, supporting the idea that yeast mtDNA (which is >25 μm) is massively condensed when packaged by nucleoid proteins [36]. This high degree of compaction is facilitated largely by Abf2 [ARS (autonomously replicating sequence)-binding factor 2], a *bona fide* mtDNA packaging protein [51] that is present at a ratio of 1 molecule per every 15-30 bp of mtDNA [48, 52, 53]. Abf2 binds mtDNA in a sequence-independent fashion but exhibits a preference for GC-rich sequences [51], and this interaction introduces sharp-angle curves (~78°) to mtDNA molecules (both linear and circular) [54]. Abf2 contains two DNA-binding domains – high mobility group (HMG) boxes, and recently it was revealed that each HMG box of Abf2 induces a sharp 90° bend in the contacted DNA, causing an overall U-turn [55]. Thus, mtDNA compaction in yeast is achieved by Abf2 via a rather simple mechanism that involves sharp bending of the DNA backbone and U-turn formation [56]. Abf2 is present at concentrations high enough to fully coat the entire mitochondrial genome [10, 52, 53] and it remains to be elucidated if other proteins are also involved in mtDNA compaction *in vivo* [56]. This non-histone HMG protein displays homology to the DNA-binding HMG proteins of nuclear chromatin [48, 57]; however, unlike the nuclear counterparts, which are known to regulate transcription and maintenance of DNA architecture, Abf2 lacks a role in mtDNA transcription and seems to be involved specifically in mtDNA packaging and protection [36, 51]. Indeed, the mtDNA in *abf2* mutants is less

protected, as evidenced by increased sensitivity to nuclease attack [58] and oxidative stress [59]. Abf2 has also been shown to bind and stabilize mtDNA-recombination intermediates [60], further highlighting the importance of this protective protein in overall mtDNA metabolism. In addition to Abf2, a multitude of other nucleoid-associated proteins exist in yeast mitochondria, including those involved in mtDNA transactions (Rim1, Rpo41, Mip1, Mgm101, Pif1, etc.), mitochondrial protein quality control (Hsp60, Ssc1, Mdj1, Mge1, etc.), and metabolism (Aco1, Atp1, Idh1, Ilv5, etc.) [36]. Interestingly, the majority of identified nucleoid-associated proteins appear to be bifunctional: for example, Aco1 and Ilv5, in addition to participating in carbohydrate and amino acid metabolism, respectively, also play an important role in the maintenance of mtDNA as nucleoid components [36, 48].

Initially, nucleoids were believed to be static; however, evidence is now accumulating in favor of their dynamic behavior, and this dynamicity has been generally linked to the metabolic status of yeast cells. For example, when cells are grown under respiring condition (with glycerol as a carbon source), the ratio of Abf2 to mtDNA decreases, and nucleoids attain a more open structure. On the contrary, when respiration is repressed (with glucose as a carbon source), nucleoids become more compact with a concomitant increase in Abf2 to mtDNA ratio. Thus, the abundance of Abf2 in nucleoids appears to determine the extent of nucleoid compaction [51]. As respiring growth medium necessitates functioning of the OXPHOS system, components of which are encoded by the mtDNA, it seems plausible that loosening of nucleoids under such condition favors mitochondrial gene expression, which, in turn, is expected to enhance respiratory activity.

3.3 Human Mitochondrial DNA

The human mitochondrial genome typically forms 5 μm double-stranded, closed-circles of one genome length consisting

of 16,569 bp [61, 62] (Figure 3). Unlike in yeast, human mtDNA exhibits a remarkable economy of the organization as the genes lack introns, and intergenic sequences are either absent or reduced to a few nucleotides [9, 62]. This gene-dense molecule harbors 37 genes, 13 of which encode essential components of the OXPHOS system and include seven subunits of complex I (*ND1*, *2*, *3*, *4*, *4L*, *5*, and *6*), one subunit of complex III (*Cyt b*), three subunits of complex IV (*COX I*, *II*, and *III*), and two subunits of complex V (*A6* and *A8*). The remaining 24 genes specify the RNA elements required for translation of those 13 polypeptides and include two rRNAs (12S rRNA and 16S rRNA) and 22 tRNAs [9].

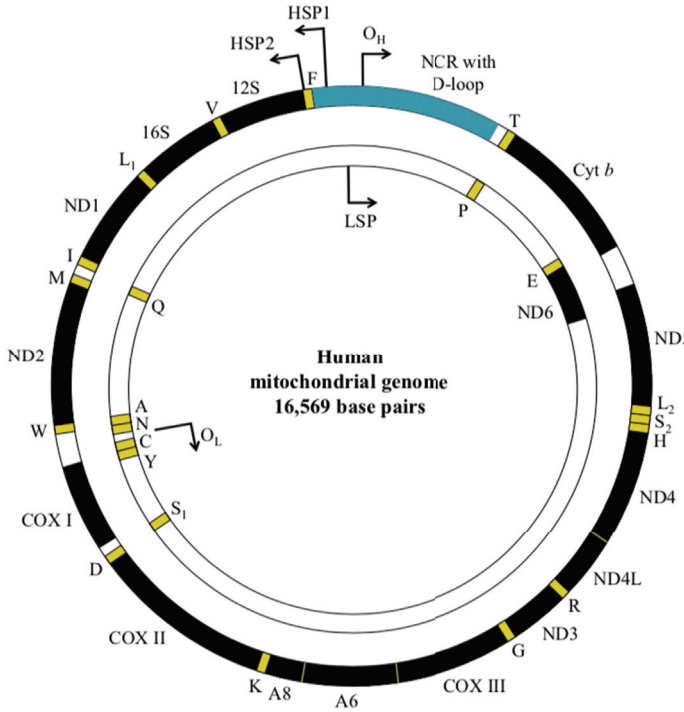

Figure 3 The map of the human mitochondrial genome. In general, the human mtDNA is a closed-circular, double helix in which the outer and inner circles designate the heavy strand and light strand, respectively. It encodes essential protein components of the OXPHOS machinery as well as RNA elements that

are required for the translation of those proteins. In the figure, black boxes depict the genes encoding 13 polypeptides and 2 rRNAs (12S and 16S), whereas yellow boxes designate the genes coding for 22 tRNAs (labeled as a one-letter amino acid symbol). The noncoding region (NCR) containing the displacement loop (D-loop) is shown as a blue box, while the arrows indicate the origins of replication and promoters for transcription. This figure is adapted from [63] and [64].

The human mitochondrial genome constitutes ~1% of cellular DNA by mass [62] and is typically maintained at a high copy number [65]. A mammalian cell typically contains 1000−10000 copies of mtDNA [66]; however, the copy number can vary tremendously depending on tissue type and the energy demands of the cell [10, 67, 68]. For example, a sperm cell can contain as little as 100 copies whereas an unfertilized oocyte can harbor hundreds of thousands of mtDNA molecules [64]. Interestingly, the content of guanine (G) plus thymine (T) in one strand of the duplex of mammalian mtDNA differs from the other strand. This difference is largest in human mtDNA and decreases gradually down the evolutionary tree. Because of this bias in nucleotide composition, the two strands of mammalian mtDNA display different buoyant densities in denaturing cesium chloride gradients such that they can be physically separated as the 'heavy' (H) and 'light' (L) strands [69-71]. The vast majority of the genetic information is localized in the H-strand that contains 28 genes (for 2 rRNAs, 14 tRNAs, and 12 polypeptides), with the L-strand possessing the remaining 9 genes (for 8 tRNAs and a single polypeptide) [9, 70]. Regulatory elements in human mtDNA are localized in two noncoding regions [62]: a small noncoding region of 30 bp harbors the L-strand origin of replication (O_L), whereas the major noncoding region, also known as NCR, spans just over a kb and concentrates almost all the noncoding DNA [61, 62]. An essential control region of mtDNA, NCR harbors the H-strand origin of replication (O_H), a single L-strand promoter (LSP), and one of two promoters of the H-strand (HSP1) [61]; a second H-strand promoter (HSP2) is

positioned downstream of HSP1 and lies within the $tRNA^{Phe}$ gene [65]. In the majority of vertebrate mtDNA duplexes, much of the NCR is occupied by a short three-stranded structure, called the displacement loop (D-loop) [69], so named because a short DNA molecule of 650 nucleotides displaces the H-strand and becomes complementary to the L-strand [61, 70, 72]. The importance of the NCR in the maintenance of the human mitochondrial genome can be validated from the observation that all partially deleted human mtDNA molecules that have been characterized retain this regulatory element [73]; even though, at least a quarter of the D-loop region seems to be expendable [74].

Human mtDNA is generally depicted as single genome-sized circles; however, its topology can vary in some cell types. As an example, mitochondria of HEK cells contain catenanes of two or more interconnected circles, which constitute up to 30% of mtDNA molecules [72, 75, 76]. Another striking example is observed in mtDNA from the adult human heart, which is partially organized in large catenated molecules with dozens of genome units containing abundant recombination and replication junctions [77]. Such complex forms of mtDNA seen in the human heart exhibit age dependency as they are absent in newborns and are progressively acquired in early childhood [78]. Moreover, this unusual mtDNA structure seems to be specific only for adult human hearts, as hearts from mice, rabbits, and pigs are devoid of such topological variation [77].

3.4 Human Mitochondrial Nucleoid

Analogous to yeast, mtDNA molecules in humans are packaged by a variety of mtDNA-binding proteins [36] to form nucleoprotein complexes that are primarily inner membrane-associated [65] and dispersed evenly throughout the mitochondrial

network [79, 80]. The number of nucleoids per cell depends largely on the cell type, and about 1,800 nucleoids are present in a human fibroblast [2]. Mammalian mt-nucleoids are slightly elongated with a mean size of 80 x 80 x 100 nm. The number of mtDNA molecules per nucleoid is 1.4 in human cells and 1.1−1.5 in mouse cells, indicating that most mammalian mt-nucleoids contain just a single copy of mtDNA [2, 79]. However, the copy number is likely to differ depending on tissue type and energy demand [81]. Supporting this notion, evidence exists in favor of the idea that multiple mtDNA molecules can be present in a single nucleoid [56, 82, 83]. Indeed, different studies have revealed that mtDNA copy number in human cells is present in between 1.4 and 7.5 per nucleoid [84]. It is important to note that an increase in mtDNA copy number results in an increase in nucleoid numbers without altering nucleoid sizes [85].

With respect to the protein components, TFAM (transcription factor A, mitochondrial) is undoubtedly the most prominent nucleoid-associated protein in human cells [72]. Like its yeast counterpart Abf2, TFAM is a member of the HMG family of proteins that plays a crucial role in the structural organization of nucleoids [86]. In addition to actively packaging DNA, TFAM exhibits cooperativity in DNA binding as its affinity for DNA increases by previously bound TFAM [86, 87]. When attached, TFAM induces a 180° bend to the mtDNA, causing a dramatic U-turn and this feature substantially contributes to a high degree of mtDNA compaction as observed in human nucleoids [80, 88, 89]. Possessing the ability to bind to any DNA sequence, TFAM can fully coat human mtDNA [80, 86, 90]. TFAM is a very abundant protein, present in about 1,000 molecules per mtDNA molecule or 1 TFAM protein molecule per 16-17 bp of mtDNA in mammalian cells [2, 56, 79, 91]. The addition of increasing amounts of pure recombinant TFAM to cloned mtDNA and subsequent imaging has

shown that TFAM, by itself, is sufficient for complete nucleoid compaction. At high TFAM/mtDNA concentrations, fully coated nucleoids, which are blocked for replication and transcription, prevail, thereby rendering nucleoid compaction by TFAM protein levels an important mechanism for control of mtDNA expression. Thus, TFAM is the only protein strictly fulfilling the criteria of a *bona fide* mtDNA packaging factor, as it fully coats mtDNA, organizes the mtDNA structure, and is indispensable for mtDNA maintenance *in vivo* [2, 92]. In addition to its role in the packaging and maintenance of mtDNA, TFAM is also critical for mtDNA transcription and replication, features that are completely absent in its yeast ortholog Abf2 [80]. In fact, TFAM was originally identified as a transcription factor involved in the assembly and promoter recognition of the mitochondrial transcription machinery [48, 93-96]. Because the initiation of human mtDNA replication depends on an RNA primer formed by transcription from LSP, the role of TFAM in mtDNA replication is also quite apparent [91]. Therefore, it is not surprising that, despite its ability to bind to any DNA sequence, TFAM has a preferred binding site upstream of mtDNA transcriptional promoters [80, 93, 97].

Apart from TFAM, the molecular composition of mammalian nucleoids is still controversial and it has been suggested that most of the proteins participating in different processes, such as replication and transcription factors, are only temporarily associated with the nucleoid [2, 56]. Nonetheless, evidence suggests that the human mt-nucleoid is composed of an array of additional proteins with diverse functions, ranging from mtDNA transaction [mitochondrial single-stranded DNA-binding protein (mtSSB), DNA polymerase gamma (pol γ), Twinkle helicase, mitochondrial RNA polymerase (POLRMT), etc.] to factors involved in protein quality control (Lon protease, HSP60, HSP70, etc.), metabolism (aspartate aminotransferase, adenosine nucleotide transferase, carbamoyl phosphate synthetase, ATPase β subunit, etc.), cellular architecture

(actin and vimentin), and signal transduction (prohibitin 1, prohibitin 2, etc.) [36, 87].

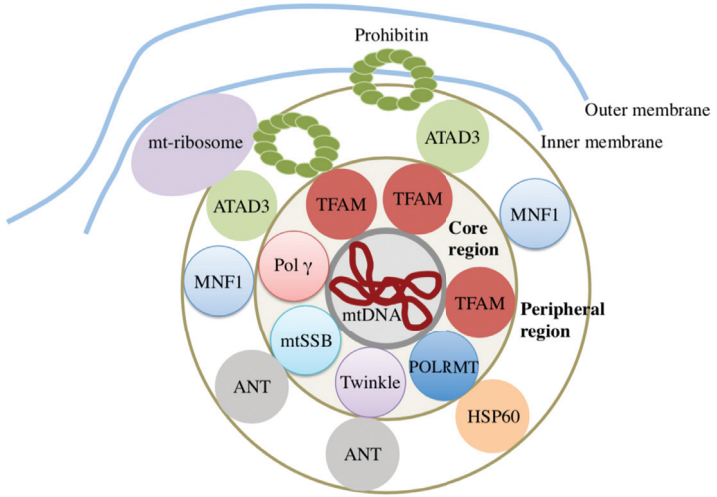

Figure 4 Layered structure of nucleoid-associated proteins. Proteins that interact directly with mtDNA are located in the core region, whereas proteins in the peripheral region interact with the proteins in the core region or with other mitochondrial proteins. ANT, adenine nucleotide translocator; ATAD3, ATPase family AAA-domain-containing protein 3; HSP60, heat-shock protein 60; MNF1, mitochondrial nucleoid factor 1; mt-ribosome, mitochondrial ribosome; mtSSB, mitochondrial single-stranded DNA-binding protein; Pol γ, DNA polymerase γ; POLRMT, mitochondrial RNA polymerase. This figure is adapted from [98].

Apparently, some of the nucleoid-associated proteins do not directly interact with mtDNA, and as an explanation, a 'layered' structure of human mt-nucleoid has been proposed (Figure 4). In this model, nucleoid-associated proteins are classified into core components that directly bind to mtDNA and peripheral components that do not interact with mtDNA but associate with the core components via protein-protein interaction. The core nucleoid proteins include those involved in mtDNA packaging, replication, and transcription (such as TFAM, pol γ, POLRMT, etc.) whereas the peripheral proteins include those involved in translation and

complex assembly (including ATAD3, prohibitin 1, prohibitin 2, etc.) [80, 87, 99, 100]. Because some of these peripheral proteins are components of nucleoids as well as the inner membrane, they are believed to serve as anchors that tether nucleoids to the inner membrane thereby ensuring even distribution of mitochondrial genetic material throughout the cell's mitochondria [99, 101, 102]. The mtDNA covered by the protein complex of the nucleoid structure has been proposed to protect it from DNA damage as it would restrict access to damaging agents. This idea is reinforced by the fact that overexpression of TFAM reduced mtDNA damage and prevented cellular dysfunction in a diabetic neuropathy animal model [11, 103].

4

MITOCHONDRIAL DNA DAMAGE

DNA damage occurs naturally within a cell. It can be caused by both endogenous agents that originate within a cell or by exogenous agents from the environment [104]. Mismatches generated during DNA replication and oxidative damage that occurs from the production of reactive oxygen species within the cell through normal metabolism are examples of endogenous damaging agents. On the other hand, exogenous factors include ionizing radiation that can produce toxic double-strand breaks (DSBs), ultraviolet (UV) radiation that can result in the formation of cyclobutane pyrimidine dimers (CPDs), and chemotherapeutic agents like cisplatin that can lead to DNA alkylation and DNA crosslinks [104, 105].

MtDNA is exposed to the same endogenous and exogenous factors as nuclear DNA (nDNA) that produce different types of damage including alkylation, adduct formation, mismatched bases, abasic (apurinic/apyrimidinic or AP) site formation, single-strand breaks (SSBs), DSBs, and oxidative lesions [10]. Estimates indicate that each cell within the human body experiences tens of thousands of DNA-damaging events per day [106, 107]. A wide range of environmental carcinogens as well as alkylating agents have been known to introduce covalent modifications preferentially to mtDNA compared to nDNA in mammalian cell cultures and

experimental animal models [108-110]. In agreement with this, different studies have shown a higher amount of lesions in mtDNA compared to nDNA in different types of cells and tissues [109, 111, 112]. The ratio of the resulting mtDNA to nDNA lesion varies from several folds for aflatoxin B_1 [113] and alkylating agents [109, 114-117], and 50 to 100-fold for peroxidation-derived DNA adducts [118]. MtDNA also contains higher amounts of different types of oxidative DNA lesions compared to nDNA [108]. Moreover, the higher replication rate of mtDNA compared to nDNA may contribute to increased levels of DNA damage [119]. Collectively, these facts underscore the importance of mtDNA as a target for both endogenous as well as exogenous DNA damaging agents [109].

Different systems are known to deal with mtDNA damage. Firstly, several DNA repair pathways can repair mtDNA lesions. Of different mtDNA repair pathways, base excision repair is extensively studied and most well understood. Secondly, the damaged mtDNA molecules can be removed via a mitochondria-specific degradation pathway to maintain mtDNA integrity. Thirdly, like mtDNA, mitochondrial deoxyribonucleoside triphosphate (dNTP) pool can also be oxidized, which can cause mismatch errors during mtDNA synthesis. To avoid misincorporation of damaged dNTPs in mtDNA, different triphosphatases sanitize the dNTP pool in the mitochondrial compartment. And finally, during mtDNA replication, pol γ or other mtDNA polymerases now reported in mitochondria can bypass certain lesions or even restart replication that is facilitated by PrimPol. It should be noted that these pathways are not mutually exclusive and can occur concurrently to deal with mtDNA damage [109].

4.1 Mitochondrial DNA Damage by Reactive Oxygen Species

Reactive oxygen species (ROS) is a general term that includes molecular oxygen-derived free radicals such as superoxide anion

radicals ($^{\bullet}O_2^-$) and hydroxyl radicals ($HO^{\bullet}$) as well as non-radical species like hydrogen peroxide (H_2O_2). Generated by single-electron reduction of molecular oxygen, $^{\bullet}O_2^-$ is widely considered as the progenitor ROS as it can lead to the formation of other ROS like $HO^{\bullet}$ and H_2O_2. Together, these molecules have the potential to inflict oxidative damage to DNA, proteins, and lipids; events that can, in turn, impair cellular functions [1].

Despite being a stable, well-protected molecule, cellular DNA can be attacked by ROS and the ensuing oxidative damage represents a major form of DNA damage confronted by aerobic organisms [62]. ROS have been known to instigate several types of DNA lesions including base modifications, loss of bases, damage to the deoxyribose sugar, SSBs, DSBs, intra- and inter-strand crosslinks, and DNA-protein crosslinks [120, 121]. These abnormalities can trigger gross structural changes in the genome that have been implicated in a plethora of diseases including cancer [121]. In addition to DNA, ROS can also attack other cellular macromolecules, such as lipids and proteins, to generate reactive intermediates that can in turn attack DNA [122]. In this regard, the polyunsaturated fatty acid residues of the phospholipid bilayer are particularly vulnerable. Following assault by ROS, these unsaturated fatty acids initially produce lipid hydroperoxides, which can, in turn, react with metals to produce a variety of mutagenic products such as malondialdehyde and 4-hydroxynonenal [122, 123].

Different ROS have a varying degree of reactivity to the DNA [124], with $HO^{\bullet}$ being the most reactive and has been largely implicated for the majority of oxidative lesions [120]. $HO^{\bullet}$ reacts with DNA by incorporating into the double bonds of bases, as well as by removing a hydrogen atom from the methyl group of T and the C-H bonds of deoxyribose [122, 125]. Because of its high reactivity, $HO^{\bullet}$ does not diffuse more than one or two molecular

diameters before reacting with a cellular component [122, 126]. Hence, to oxidize DNA, it must be generated in the immediate vicinity of the nucleic acid molecule. It is plausible that H_2O_2 acts as a diffusible, latent form of $HO^{\bullet}$, that reacts with a metal ion in close proximity to a DNA molecule to generate the oxidant [122, 127, 128]. Other species such as $^{\bullet}O_2^-$ and H_2O_2 do not seem to damage DNA at physiological concentrations; however, these less reactive species can be converted to the highly reactive $HO^{\bullet}$, which can readily assault DNA. Moreover, $^{\bullet}O_2^-$ can also combine with nitric oxide resulting in the production of peroxynitrite, an extremely potent oxidant capable of directly oxidizing DNA molecules [120].

Perhaps the most common form of DNA damage inflicted by ROS is base damage. To date, more than 20 distinct oxidized base lesions have been documented that include 8-hydroxyguanine (8-OHG; Figure 5B), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 8-hydroxyadenine, 5-hydroxycytosine, 5-hydroxyuracil, and thymine glycol (Figure 5F) [125, 129]. Because of its high electron density and low oxidation potential, G, among all DNA bases, is most frequently targeted by ROS resulting in the generation of a multitude of toxic products [121, 129], of which 8-OHG is the most commonly produced base lesion [124]. For this reason, 8-OHG is widely accepted as the biomarker for oxidative DNA damage [124, 129]. While damages to DNA can occur following its interaction with ROS, oxidation of free dNTPs in the nucleotide pool also accounts for oxidative damage. These oxidized dNTPs can be incorporated into the genome by various replicating and repair DNA polymerases [130-132]. Most oxidative base lesions are mutagenic, regardless of whether they are generated *in situ* or occur by misincorporation from the dNTP pool [125]. As an instance, 8-OH-deoxyguanosine formed *in situ* can result in G to T substitutions, whereas misincorporation of 8-OH-deoxyguanosine

triphosphate opposite deoxyadenosine can produce adenine (A) to cytosine (C) substitutions [125, 133].

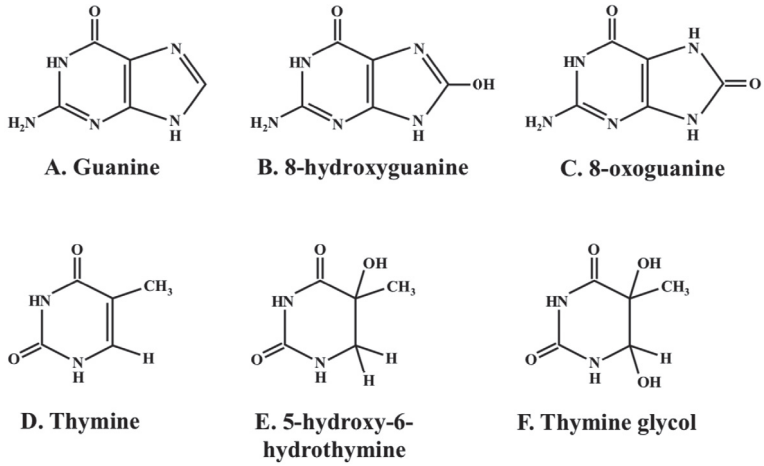

Figure 5 Structures of normal and oxidized DNA bases of guanine and thymine.

Damage to DNA by ROS occurs naturally as low steady-state levels of oxidative base lesions have been identified *in vivo* both in nDNA as well as in mtDNA [124]. In the context of the mitochondrial genome, the primary products of ROS-induced DNA damage are 8-oxoguanine (8-oxoG, an oxidation product of 8-OHG [121]) (Figure 5C) among purines [134-136] and thymine glycol among pyrimidines [137]. Possible pathological consequences of ROS attack include the formation of mutations, strand breaks, and deletions (due to misrepair of breaks or damage) to the mtDNA, oxidative damage to the ETC components, lipid peroxidation, and overall mitochondrial dysfunction [138, 139]. Such alterations can be detrimental to cells as persistent damage to mtDNA has been known to impair mitochondrial function, induce permanent growth arrest, and commit cells to apoptosis [140]. It is interesting to note that oxidative DNA base damage (measured as 8-OHG) has been identified in mtDNA at steady-state levels several-fold higher than

in nDNA [124, 141-143]. It has been demonstrated that mtDNA damage is more extensive and persists longer than nDNA damage in human cells following oxidative stress [140]. MtDNA has also been revealed to be damaged at lower ROS levels than nDNA [8]. The increased vulnerability of mtDNA to ROS could be due to several factors, including (i) the close proximity of mtDNA to the sites of ROS production at the inner membrane of the mitochondria, which makes mtDNA 10 to 20-fold more susceptible to damage compared to its nuclear counterpart [104, 144, 145]; it should be noted that approximately 90% of total cellular ROS are generated in the mitochondria during ATP synthesis through OXPHOS [138, 146]; (ii) mtDNA, unlike its nuclear counterpart, is not condensed into complex nucleosomes and hence is more susceptible to damage by ROS generated during OXPHOS [104, 140]; (iii) mitochondria have reduced complement of DNA repair pathways; in addition, repair of mtDNA is a slower process than nDNA, especially after longer durations of oxidative stress [8]; and (iv) there is a high prevalence of localized metal ions (such as Fe^{2+}) in the mitochondrial compartment that may serve as catalysts for ROS generation [140]. Moreover, the association of mtDNA molecules with the inner membrane is also believed to contribute to the overall damage as reactive intermediates formed during membrane lipid peroxidation have been suggested to attack the mitochondrial genome [124, 147]. Interestingly, a study has demonstrated that oxidative stress can lead to the degradation of mtDNA molecules and that strand breaks and AP sites prevail over mutagenic base lesions in ROS-damaged mtDNA. The higher inclination of ROS to instigate mtDNA strand breaks and AP sites as opposed to mutagenic base lesions may be a novel mechanism adopted by mitochondria to maintain genomic integrity as a higher degree of lesions to the sugar-phosphate backbone induces degradation of mtDNA, thereby preventing the accumulation of mutagenic base lesions [148].

4.2 Mitochondrial DNA Damage by Ionizing Radiation

Ionizing radiation (IR) is one of the major cancer treatment regimens that kills cells in the exposed tumor tissues primarily by introducing DNA damage [149]. The effect of IR on macromolecules can be either direct or indirect. It has been estimated that about one-third of DNA damage results from its direct effect [150] that occurs when IR deposits energy directly into the biomolecule [150, 151]. This results in disruption of the atomic structure of the biomolecule, initiating a chain of events that drives chemical and biological alterations [150, 151]. Alternatively, IR can also act indirectly via radiolysis of water, a phenomenon responsible for the remaining two-third of DNA damage [150]. The absorption of energy by water, an abundant cellular constituent, results in its excitation as well as ionization that culminates in the generation of excessive ROS [150]. In an aerobic cellular environment, the major ROS produced by the indirect effect include $HO^{\bullet}$, $^{\bullet}O_2^-$, and H_2O_2 [150]. It was initially thought that the biochemical changes that occur during or immediately following irradiation were, by and large, responsible for the adverse effects of the IR. However, oxidative modifications may transpire even after days and months following the initial IR exposure presumably because of continual production of ROS and reactive nitrogen species, and these phenomena are believed to contribute significantly to the deleterious effects of IR [150, 152]. Therefore, it is not surprising that exposure to IR results in DNA lesions that are, in the main, chemically identical to those generated by ROS [149, 153-155], with base damages being most prevalent, followed by SSBs and DSBs [149, 153]. Estimates have revealed that one gray of gamma radiation induces about 850 pyrimidine lesions (predominantly thymine glycol), 450 purine lesions (predominantly FapyG and 8-oxoG), 1000 SSBs, and 20-40 DSBs per mammalian cell [149, 153]. Because of its cytotoxicity, the most deleterious lesion

instigated by IR is believed to be the DSB, which consists of a break in the phosphodiester backbone of both strands of the DNA separated by about 10 bp or less [149, 156, 157]. The level of DSBs is directly proportional to the radiation dose, starting from a dose of a few milligray [149, 158]; moreover, the more relaxed the DNA is (e.g., transcriptionally active DNA) the level of the DNA damage also increases and the damage becomes more complex [149, 159, 160]. In addition, IR is also notorious for instigating clustered damage, which contains two or more lesions within one or two helical turns of DNA [149]. Indeed, cluster lesions are widely considered as the hallmark of IR-induced damage, which is in contrast to endogenously induced lesions that are more isolated and tend to be distributed homogenously in the DNA [149]. As with DSBs, the energy deposited by IR is directly proportional to the complexity as well as yield of clustered lesions [149, 161]. Because clustered damaged sites, particularly those comprising DSBs, are structurally and chemically complex, these lesions have reduced reparability compared to individual isolated lesions, and this is believed to be one of the main reasons why IR is more effective in killing tumor cells [149].

It is believed that damage to nDNA is the primary cause of the deleterious effects of IR [162, 163]. However, a variety of lesions similar to those found in nDNA have also been identified in mtDNA following IR exposure [162]. In fact, mtDNA has been known to suffer more from IR-induced DNA lesions compared to its nuclear counterpart, a phenomenon analogous to the increased vulnerability of the mitochondrial genome to ROS [162]. Moreover, the human mtDNA is also known to undergo the so-called "common deletion" following IR exposure [164] that involves the loss of 4977 bp from mtDNA. Because it includes genes encoding essential components of the ETC and OXPHOS, the common deletion has been proposed to result in ineffective mitochondrial metabolism

with a concomitant increase in mitochondrial ROS production [150, 165]. These observations indicate that mtDNA may play a significant role in translating radiotoxic effects of IR, an idea greatly substantiated from the fact that human rho zero (ρ^0; lacking mtDNA; respiratory-deficient) cells are more resistant to radiation-induced cell killing compared to the rho positive (ρ^+; containing wild-type mtDNA; respiratory-proficient) counterparts [150, 166]. Furthermore, IR is also known to trigger alteration of mitochondrial functions [162, 167, 168], escalation of mitochondrial oxidative stress [162, 169-172], and induction of apoptosis [162, 173-176]. In fact, mitochondria have been reported to be the primary target for radiation-induced apoptosis [162, 177]. Taken together, it would not be irrational to state that mitochondria are major targets of IR, in addition to the cell nucleus.

4.3 Mitochondrial DNA Damage by Chemotherapeutic Agents

As another major component of anticancer therapy, chemotherapeutic drugs are being extensively exploited to eliminate cancer cells, diminish tumor growth, and alleviate pain. DNA damaging chemotherapeutic agents react chemically with DNA to alter DNA bases, intercalate between DNA bases, or induce DNA crosslinks. As an example, nitrogen mustard derivatives (e.g., cyclophosphamide) act by directly alkylating purine bases [178], which can subsequently result in the formation of G-G and G-A interstrand crosslinks within the DNA double helix [179]. Nitrosoureas (e.g., carmustine) are another type of DNA-alkylators that, like nitrogen mustards, alkylate DNA bases and induce interstrand crosslinks [178]. Likewise, certain antitumor antibiotics like mitomycin C also have similar dual effects [178, 179]. Base alkylation is known to block replication fork progression, which can also be triggered by DNA crosslinks. Thus, the presence of both types of lesions is believed to have a more potent effect on cancer cells compared to a single type of damage, and such lesions, if not

repaired, can lead to cell death via apoptosis [178]. Anthracycline antibiotics (e.g., doxorubicin), another type of antineoplastic agents, exhibit multiple mechanisms of action apart from the ability to alkylate bases and induce DNA crosslinks. These include the ability to intercalate into DNA, produce free radicals, interfere with helicase activity, poison topoisomerase II, and induce apoptosis thereby making these drugs extremely cytotoxic [178, 180]. Other DNA damaging chemotherapeutic agents with substantial impact against cancer cells include alkylating-like platinum agents (e.g., cisplatin) [178] and glycopeptide antibiotics such as bleomycin [181]. In fact, cisplatin is regarded as one of the most important anticancer drugs ever developed. Following its entry into the cell, cisplatin is hydrated to produce a positively charged species that can interact with nDNA as well as other nucleophilic biomolecules within the intracellular milieu [182, 183]. Cisplatin, an inorganic molecule containing a platinum core, binds to purine bases, particularly to G residues and, to a lesser extent, A residues to form adducts on DNA [178]. When two platinum adducts form on nearby bases, either on the same strand or opposite strands, the formation of intra- or interstrand crosslinks ensues [178, 179]; however, intrastrand crosslinks between adjacent G residues have been regarded as the most prevalent form of DNA damage induced by cisplatin [179]. These different adducts and crosslinks formed by cisplatin have various effects on a cell including DNA unwinding, DNA bending, and hindering DNA replication and transcription, which can result in DNA strand breaks [179, 184]. The genotoxicity induced by cisplatin has been attributed to its ability to produce intra- and interstrand crosslinks in the nDNA. However, estimates suggest that only ~1% of intracellular platinum is bound to nDNA, with the vast majority of the drug available for interaction with other intracellular nucleophilic sites on other molecules including mtDNA [182, 185, 186]. Indeed, it has been demonstrated that cisplatin accumulates in mitochondria [187]. Owing to the

electrochemical gradient across the mitochondrial inner membrane, the mitochondrial matrix acquires a negative charge, which is thought to electrophoretically pull and cause the accumulation of the positively charged cisplatin [182]. In agreement with this concept, studies have indicated that mtDNA-cisplatin adducts may be significantly more common than nDNA-cisplatin adducts in the same cell line treated with the same concentration of cisplatin [182, 188, 189]. For example, treatment of cisplatin to head and neck squamous cell carcinoma (HNSCC) revealed 300- to 500-fold more platinum adducts in mtDNA than in nDNA. Interestingly, when HNSCC cytoplasts (viable and functional cell bodies lacking nuclei) were treated with cisplatin, they retained dose-dependent cisplatin sensitivity similar to the parental cells. On the other hand, HNSCC ρ^0 cells were 4- to 5-fold more resistant to cisplatin compared to the parental cells. Moreover, cisplatin also triggered the rapid release of cytochrome *c* from mitochondria and induced marked mitochondrial disruption characteristics of permeability transition pore opening [190]. These observations corroborate the idea that mitochondria are the major target for cisplatin in cancer cells [190, 191].

Bleomycin is another important anticancer drug that is believed to participate in damaging both nDNA as well as mtDNA. Bleomycin requires specific cofactors (a reduced transition metal, oxygen, and a one-electron reductant) to generate 'activated' bleomycin, which can oxidize lipids [192], hydrolyze amide bonds of proteins [193], and induce strand breaks in both DNA [194] as well as RNA [195]. The activated bleomycin can decompose to produce $HO^{\bullet}$, which can react rapidly and non-specifically with any molecule it encounters. Nonetheless, a wide array of experimental evidence indicates that the cytotoxicity of bleomycin is principally due to its DNA damaging activity. Bleomycin is a positively charged molecule and hence can bind to DNA electrostatically

[181]. Recently, experiments have demonstrated that bleomycin preferentially damages mtDNA compared to nDNA, and this was accompanied by a concomitant reduction in mitochondrial function as well as basal oxygen consumption. Moreover, experiments with cytoplasts from TEX cells revealed that these nuclei-lacking cell bodies were equally sensitive to the same concentrations of bleomycin as whole cells, whereas ρ^0 Jurkat cells were significantly more resistant to bleomycin compared to the isogenic wild-type counterparts [196]. Likewise, experiments with human alveolar epithelial cells also revealed that bleomycin induced more damage to mtDNA compared to nDNA, and ρ^0 human alveolar epithelial cells were more resistant to bleomycin toxicity compared to the parental wild-type cells [197]. Thus, it seems likely that bleomycin-induced cytotoxicity occurs primarily because of its effect on mtDNA.

5
MITOCHONDRIAL DNA REPAIR

In a typical cell, mitochondria are the major sites for ROS generation and to deal with these metabolic byproducts, mitochondria are equipped with comprehensive antioxidant defense systems. However, deficiency or inefficiency of these ROS detoxifying systems could be deleterious for the integrity of the mitochondrial genome as several kinds of mtDNA damage can arise from ROS exposure [62]. As mtDNA encodes essential components of the ETC and OXPHOS, failure to repair mtDNA lesions can lead to disruption of the ETC and enhanced ROS generation, which can, in turn, result in energy depletion and ultimately cell death [198-200]. Thus, an efficient mtDNA repair system is indispensable for maintaining the integrity of the mitochondrial genome and consequently for cellular homeostasis [198].

DNA repair was first reported for the nuclear compartment and was originally believed to confine only to the nucleus [201]. Due to the multi-copy nature of the mitochondrial genome, it was initially thought that DNA repair mechanisms might not be required in mitochondria [10]. As mitochondria were not capable of removing CPDs formed following UV-irradiation [202, 203], it was believed for many years that these organelles lack their own DNA repair systems [11, 81, 198]. Lesions from UV-damage

are commonly repaired by nucleotide excision repair (NER), a pathway that has not been identified in mitochondria to date [11]. Therefore, for many years it was thought that the integrity of the mitochondrial genome was preserved simply by degrading damaged molecules, rather than repairing them [10, 201]. However, it is now becoming clear that most DNA repair pathways that are present in the nucleus also exist, at least partially, in mitochondria of several model organisms, including humans [11]. These repair pathways, together with controlled degradation of mtDNA, help maintain the integrity of the mitochondrial genome [198, 201, 204].

The mitochondrial genome does not encode DNA repair proteins and these organelles rely on the translocation of nuclear-encoded repair enzymes, that, in general, localize to the inner membrane in the form of mt-nucleoids [201]. The enzymes accountable for mtDNA repair are, in most instances, encoded by the same genes as their nuclear counterparts [205]. So, many mitochondrial repair pathways make use of proteins with dual nuclear as well as mitochondrial localization, and, consequently, many mitochondrial repair pathways are reminiscent of those found in the nucleus [10]. The basic principles of repair of nDNA and mtDNA also seem to be similar with the major difference being nDNA repair pathways involve a larger number of proteins compared to the mitochondrial counterparts. Moreover, our knowledge of mtDNA repair pathways is limited in comparison to nDNA repair pathways [206]. Whilst nDNA is safeguarded by repair-oriented maintenance, mtDNA can either be repaired or degraded, a choice facilitated by the multi-copy nature of these molecules. The selective degradation of damaged mtDNA reduces the overall population of mutated and/or deleted mtDNA molecules. It has been shown that mtDNA molecules containing AP sites [207-209] or DSBs [207, 210, 211] could be directly

degraded rather than repaired. Because mtDNA is under strict copy-number control, selective degradation of damaged mtDNA molecules instigates replication of the undamaged mtDNA copies thereby reconstituting the mtDNA pool [212]. Apart from selective degradation, damaged mtDNA molecules can also be repaired [207]. In sum, the nucleus and mitochondria share a number of factors and processes involved in DNA repair; however, DNA repair in mitochondria also exhibits specific and original aspects [201].

DNA repair proteins are often mentioned to accumulate in the cytosol under unstressed conditions and then translocate into mitochondria in response to DNA damage [201]. For example, yeast cells subjected to nuclear or mitochondrial oxidative stress revealed that Ntg1, an enzyme with DNA glycosylase/AP lyase activity, relocalizes dynamically to the respective subcellular compartment on which the stress was applied. By comparing ρ^+ and ρ^0 yeast cells, it was demonstrated that the localization of Ntg1 to mitochondria was triggered by mtDNA damage and not by mitochondrial ROS generated after treatment with extrinsic agents [213]. Translocation of a pool of repair proteins from the cytosol to the damaged compartment is faster for cells than inducing *de novo* gene expression and seems to be a major regulatory mechanism of organelle DNA repair [201].

5.1 Types of Mitochondrial DNA repair

To preserve the integrity of the mitochondrial genome, cells have evolved an intricate network of mtDNA repair pathways/mechanisms, which are broadly categorized into four major classes (Figure 6). These repair pathways are direct repair/direct reversal (DR), base excision repair (BER), mismatch repair (MMR), and double-strand break repair (DSBR).

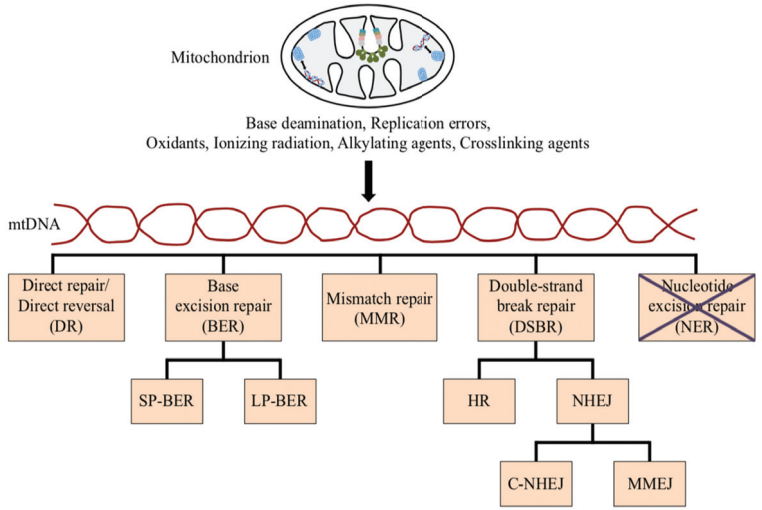

Figure 6 An overview of mtDNA repair pathways. Base deamination, replication errors, and DNA damaging agents such as oxidants, ionizing radiation, alkylating agents, and crosslinking agents can damage mtDNA. BER is the major mtDNA repair pathway. DR also occurs in mitochondria whereas the organelles are devoid of the NER pathway. Other repair pathways such as MMR and DSBR may operate in mitochondria. C-NHEJ, classical non-homologous end joining; HR, homologous recombination; LP-BER, long-patch base excision repair; MMEJ, microhomology-mediated end joining; NHEJ, non-homologous end joining; SP-BER, short-patch base excision repair. This figure is adapted from [201] and [214].

BER was the first pathway to be discovered in the mitochondria [215], and for many years, it was believed that the mtDNA repair system was limited only to this pathway [104, 204], specifically to short-patch BER (SP-BER) [198, 216]. However, the mitochondrial genome harbors a wide range of complex lesions and also has the potential for erroneous replication [198, 217]. Logically, just SP-BER seemed insufficient to handle a variety of lesions that mtDNA incur and it was speculated that additional repair pathways exist in the mitochondrial compartment. In accordance with the postulation, several studies have revealed the presence of mitochondrial long-patch BER (LP-BER) [218-221]. Evidence is also accumulating in favor of the existence of DR in

mitochondria of different species [222-226]. While components of MMR and DSBR pathways have also been identified in the mitochondria that likely function in mtDNA repair [62, 104], it is still unclear whether mitochondria possess efficient MMR and DSBR activities [207]. Even though there is a general consensus that NER does not operate in mitochondria, proteins participating in nuclear NER are found within the mitochondrial compartment [227-230].

5.1.1 Direct Repair/Direct Reversal

The simplest way to repair DNA damage is by DR, in which a single enzyme restores the damaged base without cleaving the base or the phosphodiester bond. DR enzymes are present in all domains of life, including some viruses [11]. DR has been shown for UV-induced photolesions i.e., CPDs and pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs), through photolyases. Moreover, this repair mechanism has also been demonstrated for alkylation damage (mostly methylations) through the activities of alkyltransferases for *O*-alkylated DNA bases and the AlkB family of dioxygenases for *N*-alkylated bases [231]. Enzymes participating in DR are discussed ahead.

5.1.1.1 Photolyases

Sunlight, which contains mutagenic UV-radiation, can induce the formation of DNA lesions. UV-radiation can be classified into three major types depending on the measure of their wavelength. The most dangerous, UVC (100-279 nm) is completely absorbed by the ozone layer and the atmosphere. Only UVB (280-314 nm) and UVA (315-399 nm) can both affect health under natural conditions [232]. The indirect effects of UVB and UVA include the production of ROS, which promote the formation of oxidative lesions and breaks in the DNA backbone [233]. The most significant direct

effect of UVB is the formation of covalent linkages between two adjacent pyrimidines, primarily CPDs and to a lesser extent 6-4PPs [234]. The bridging of two pyrimidines within the same DNA strand causes significant distortion of the DNA helix [235]. Such distortion blocks the progression of enzymes moving along the DNA and can result in DSBs during the collision of the replication machinery with a blocked transcription elongation complex [236]. Pyrimidine dimers can be repaired either directly by photoreactivation or indirectly by the excision repair pathways. Photoreactivation restores the structure of the DNA helix without removing the modified nucleotides. This process is performed by photolyases, enzymes that bind to the dimers and use the energy of blue light or UVA to cleave bonds between neighboring pyrimidines directly [237]. Photolyases are lesion-specific, with CPD photolyases repairing only CPDs and 6-4PP photolyases only pyrimidine-pyrimidone products. Photolyases are found in most organisms, but not in placental mammals [11]. Mitochondrial localization of photolyases has been documented in yeast and plants. In *S. cerevisiae*, a photolyase encoded by *PHR1* is targeted to mitochondria, where it directly reverses UV-radiation-induced mtDNA damage [222]. Likewise, a nuclear CPD photolyase has been identified in rice mitochondria and photoreactivation of CPDs has been shown to occur in rice mtDNA [223]. In a small flowering plant *Arabidopsis thaliana*, a CPD photolyase AtCRY3 localizes to mitochondria [238]. This enzyme is active against CPDs present in single-stranded DNA (ssDNA) [239] as well as double-stranded DNA (dsDNA) [240]. Moreover, a 6-4PP photolyase AtUVR3 was recently found to localize to mitochondria of *A. thaliana* [241]. Even though a report suggested the existence of photolyase activity in *Xenopus laevis* mitochondria [225], it is usually believed that mitochondria in higher eukaryotes do not harbor photolyase activity [204]. Nonetheless, it should be noted that two mouse photolyase-like genes have been cloned, viz., mCRY1 (mPHLL1) and mCRY2 (mPHLL2), of which mCRY1

localizes to mitochondria [242]. CRY1 has been determined as a component of the mammalian circadian clock [243]; however, a specific role in the repair of photolesions has not been shown [11].

5.1.1.2 O^6-Methylguanine DNA Methyltransferase

Among a wide range of lesions produced by alkylating agents, O^6-methylguanine is a highly mutagenic modification, which appears to be repaired almost exclusively by DR catalyzed by O^6-methylguanine DNA methyltransferase (MGMT). MGMT is a suicide enzyme that receives the methyl group in a catalytic cysteine residue, thereby restoring the G base [244]. This enzyme is often found mutated in tumors and is also associated with chemotherapy resistance in glioblastomas [245]. MGMT is the main DR enzyme in the nucleus of mammalian cells and there are data indicating that a variant of this protein may also work in mitochondria. Mitochondrial extracts from rat liver cells reveal that O^6-methyl-2′-deoxyguanosine exists in mtDNA and that this DNA lesion is effectively removed from mtDNA with kinetics similar to nDNA, whereas O^6-butyl-2′-deoxyguanosine (O^6-BudG) is not removed [117]. As O^6-BudG is removed by NER in the nucleus and not by MGMT, the lack of removal of this lesion is in accordance with MGMT activity. A separate study revealed that O^6-ethyl-2′-deoxyguanosine is removed from mtDNA from rat liver cells at rates comparable to those observed in nDNA [246]. A subsequent study revealed that mtDNA damage induced by methylating agents is repaired, whereas complex alkylation damage is not [247], further corroborating results from previous studies. These results indicate that a DR activity, corresponding to MGMT-directed repair in the nucleus, likely exists in mammalian mitochondria that removes methyl and ethyl groups, but not large alkyl groups, from the O^6-position of G. Data also indicate that a protein with the molecular weight of MGMT localizes to the mammalian mitochondrial matrix [117]. However, this report of

the presence of MGMT in mitochondria has not been corroborated, and investigators from an independent study were unable to detect MGMT in mitochondria by utilizing the Western blotting technique [248]. In addition, this protein seems to be devoid of a typical N-terminal cleavable mitochondrial targeting sequence (MTS) [244]. Nonetheless, targeting MGMT to mitochondria by fusing an MTS has revealed that mitochondrial localization of MGMT can protect human cells from alkylation damage and alkylation-induced cell death [248, 249]. It should be noted that alkylation damage present in mammalian mtDNA may also be repaired by the action of the alkyladenine DNA glycosylase (AAG) via the BER pathway [11]. Taken together, the presence of MGMT-directed repair in mammalian mitochondria remains to be clarified.

5.1.1.3 AlkB Homologs

The AlkB family of dioxygenases excises alkyl adducts from bases in an iron and α-ketoglutarate dependent oxidative dealkylation. Acting both in DNA and RNA repair with a wide variety of methylated purines as substrate, these enzymes are ubiquitous and conserved from bacteria to mammals [250]. Even though nine mammalian homologs of prototypical *Escherichia coli* AlkB have been identified, not all have been revealed to have DNA repair activity. Among these, ALKB1 (hABH1) has been shown to localize to mitochondria of human cells. *In vitro* assays with recombinant ALKB1 revealed a demethylase activity on 3-methylcytosine (3-MeC) in both ssDNA and RNA, indicating the involvement of the protein in DNA repair of alkylation damage and perhaps also in regulating levels of 3-MeC in rRNAs and tRNAs [251]. Even though no other AlkB homolog has been conclusively shown to localize to mitochondria, *alkbh7*$^{-/-}$ mice lacking the ALKBH7 protein accumulate more mtDNA lesions compared to their wild-type counterparts, indicating that ALKBH7 could also be involved in mtDNA repair [252].

5.1.2 Base Excision Repair

BER is an evolutionarily conserved pathway from bacteria to humans [11]. It is the predominant mechanism dedicated to the removal of damaged DNA bases that do not significantly distort the overall structure of the DNA double helix [106]. These non-bulky lesions inflicted to individual bases include oxidation, alkylation, deamination, and methylation [10, 253-255]. These types of damages originate from exogenous (radiation, chemotherapeutic agents, etc.) as well as endogenous sources (ROS). As mtDNA lies in close proximity to the ETC, which is the major ROS generating factory, mtDNA is more prone to ROS damage, and thus an efficient BER pathway is necessary for mitochondria [10].

Mitochondria are endowed with proficient BER, which is the main mtDNA repair mechanism in these organelles [11, 62, 204, 206]. It is also the most studied mtDNA repair pathway and remains the best characterized [11]. BER in the mitochondria is believed to occur at the inner membrane where mtDNA is compacted into nucleoids [104]. Mitochondrial expertise in this pathway is indeed logical given that mtDNA encounters a high degree of oxidative damage compared to the nuclear counterpart [204]. Evidence indicates that 8-oxoG, the most predominant oxidative DNA base lesion, is more efficiently repaired in mitochondria compared to the nucleus [256]. Even though the molecular players in nuclear BER appear to be more diverse compared to the mitochondrial counterpart, the basic steps of BER seem to be the same in the nucleus and mitochondria [204]. Accordingly, mitochondrial BER can be divided into five steps, viz., (i) recognition and removal of damaged base; (ii) strand cleavage of the AP site; (iii) DNA end processing; (iv) gap-filling; and (v) ligation. Enzymes involved in each of these steps have been identified in mitochondria making BER the only comprehensively delineated mtDNA repair pathway [62].

As with any DNA repair system, BER commences with the recognition of the DNA modification. This step is performed by a family of enzymes called DNA glycosylases [257]. DNA glycosylases recognize and remove damaged DNA bases by excising the N-glycosidic bond between the base and its corresponding deoxyribose [106]. In humans, there are 11 known DNA glycosylases, each accountable for the recognition and excision of a subset of DNA modification, although with some overlap in the substrates they detect [11]. For example, 8-oxoG is excised mainly by 8-oxoguanine DNA glycosylase (OGG1) but can also be removed by endonuclease III-like protein 1 (NTH1) [10]. This redundancy might be the reason why they are the only components of the pathway that, when deleted, do not result in embryonic lethality in mice. Nuclear genes encode all DNA glycosylases and some of these contain an MTS that permit translocation to the mitochondria [104, 206, 258]. Except for SMUG1, TDG, MBD4, and NEIL3, all DNA glycosylases previously identified in the nucleus, have been shown to localize to mitochondria [259] (Table 1). In several instances, the mitochondrial isoforms of the various DNA glycosylases are distinct from their nuclear counterparts. For example, the human *UNG* gene encodes two main isoforms of the uracil DNA glycosylase (UDG), uracil-N-glycosylase 1 (UNG1; mitochondrial) and uracil-N-glycosylase 2 (UNG2; nuclear) [260], which are produced by alternative splicing and transcription from different positions in the *UNG* gene [261]. The MutY homolog DNA glycosylase (MUTYH) has ten isoforms, produced by alternative splicing of its three major transcripts: α, β, and γ. It is believed that the mitochondrial isoform arises from the α1 transcript, whereas the nuclear isoforms from β1, β3, or γ2 transcripts [11]. As for methylpurine DNA glycosylase (MPG), three transcriptional isoforms have been identified: A, B, and C. Of these at least A and B have a putative MTS and have been detected in mitochondria [262]. In the case of the human

OGG1 gene, seven alternatively spliced mRNAs were identified, with two main polypeptides OGG1α being nuclear as well as mitochondrial, and OGG1β being exclusively mitochondrial [263]. On the other hand, only one isoform has been identified for the mouse OGG1. For NTH1, endonuclease VIII-like glycosylase 1 (NEIL1) and 2 (NEIL2), their mitochondrial localization and activity have been determined in mitochondrial extracts [258, 264, 265]; however, their mitochondrial isoforms have not been thoroughly characterized.

Table 1 The localization and mechanistic details for all known human DNA glycosylases. This table is adapted from [11].

DNA glycosylase	Mitochondrial localization	Type	AP-lyase activity	3′ End	5′ End	3′ Processing	5′ Processing
SMUG1	−	M	−	3′-OH	5′-dRP	−	Pol β
TDG	−	M	−	3′-OH	5′-dRP	−	Pol β
MBD4 (MED1)	−	M	−	3′-OH	5′-dRP	−	Pol β
UNG (UDG)	+	M	−	3′-OH	5′-dRP	−	Pol β or Pol γ
MUTYH (MYH)	+	M	−	3′-OH	5′-dRP	−	Pol β or Pol γ
MPG (AAG)	+	M	−	3′-OH	5′-dRP	−	Pol β or Pol γ
OGG1	+	M/B	β-lyase	3′-UHA	5′-PO_4^-	APE1	−
NTHL1 (NTH1)	+	B	β-lyase	3′-UHA	5′-PO_4^-	APE1	−
NEIL1	+	B	β/δ-lyase	3′-PO_4^-	5′-PO_4^-	PNKP or APE1	−
NEIL2	+	B	β/δ-lyase	3′-PO_4^-	5′-PO_4^-	PNKP or APE1	−
NEIL3	−	M/B	β/δ-lyase	3′-PO_4^-	5′-PO_4^-	PNKP or APE1	−

3′-UHA, 3′-unsaturated hydroxyaldehyde; B, bifunctional; M, monofunctional; M/B, can act either as monofunctional or bifunctional.

DNA glycosylases utilize a base-flipping mechanism to detect and excise the altered base that depends on the stability of the unmodified base paring that will not flip out of the double-strand. However, the pairing is not optimal with a modified base, and the enzyme manages to flip the base, which is then placed in the catalytic site of the DNA glycosylase [266, 267]. DNA glycosylases can either be monofunctional or bifunctional (Table 1), depending on whether they possess an intrinsic lyase activity

[104]. Monofunctional glycosylases remove the damaged base but are devoid of lyase activity [104]. Following removal of damaged bases, an AP site is generated and monofunctional glycosylases are dislodged from the AP site by apurinic/apyrimidinic endonuclease 1 (APE1), which catalyzes the hydrolysis of the phosphodiester linkage immediately 5′ to the AP site, thereby generating a SSB with a 3′-hydroxyl (OH) group on one end, and a 5′-deoxyribose phosphate (dRP) group on the other (Figure. 7). Bifunctional glycosylases, on the other hand, also possess an intrinsic lyase activity that nicks phosphate backbone 3′ to the lesion following removal of the damaged base [104]. The activity of bifunctional enzymes generates strand ends that will require some processing before proceeding further in the BER pathway [11].

Different DNA glycosylases create different types of 3′ and 5′ ends, which necessitate further processing to produce the 3′-OH and 5′-phosphate termini required for polymerization and ligation. For monofunctional glycosylase, APE1 functions as the endonuclease in the nucleus and mitochondria [268] that leaves a 3′-OH and 5′-dRP termini. 5′-dRP is further processed by a DNA polymerase via its phosphodiesterase (dRPase) activity that removes the 5′-dRP to produce 5′-phosphate. Pol γ possesses the 5′-dRP lyase activity and can support mitochondrial BER *in vitro* [269]. In addition, mitochondrial localization of DNA polymerase beta (pol β) and its role in mitochondrial BER has also been shown [270, 271]. When the damage is removed by a bifunctional DNA glycosylase with β-lyase activity (e.g., NTH1), the enzyme produces a 3′ end with an unsaturated hydroxyaldehyde (UHA) and a 5′-phosphate [254]. The processing of the 3′ end is achieved by the phosphodiesterase activity of APE1, producing a 3′-OH. On the other hand, bifunctional glycosylases with β/δ-lyase activity (e.g., NEIL1) will produce a 3′-phosphate and a 5′-phosphate

[254]. The 3′-phosphate can be excised by the polynucleotide kinase/phosphatase (PNKP), which possesses both 5′ kinase and 3′ phosphatase activity. 3′-phosphate can also be removed by APE1, even though its phosphatase activity is not that robust [11, 272].

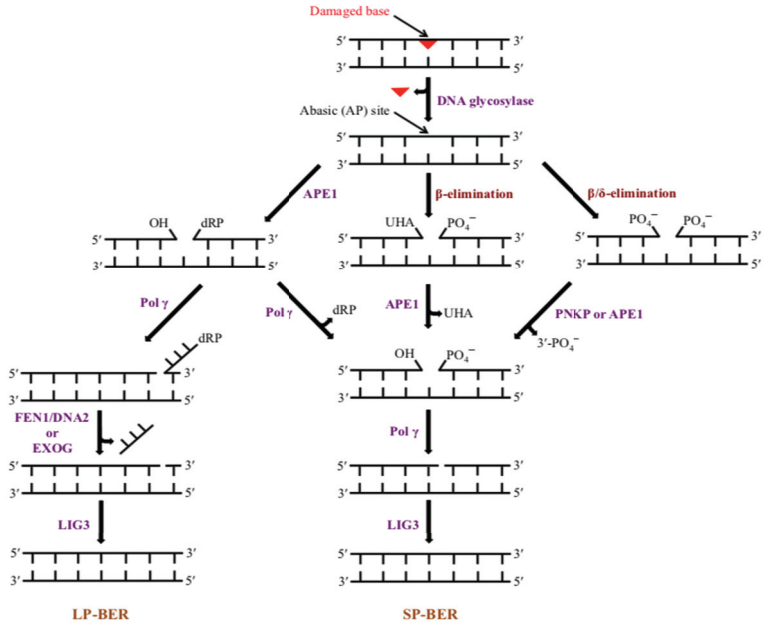

Figure 7 Schematic representation of the BER pathway. The figure emphasizes the coordinated steps and catalytic components identified in SP-BER and LP-BER in mtDNA. The initial base damage is shown in red. This figure is adapted from [11].

After proper end processing, repair can be accomplished by nucleotide insertion and ligation. Both replicative mitochondrial polymerases pol γ [269] and pol β [270, 271] can support BER nucleotide incorporation in mammalian mitochondria; however, the mechanism determining polymerase choice or the relative contribution of each polymerase for mitochondrial BER is still unclear [11]. At this point, BER in mitochondria can proceed either through SP-BER or LP-BER. Following strand cleavage, certain 5′-groups (e.g., 5′-oxidized dRP) become resistant to the lyase

activity of pol γ [204] due to which the 5′-end becomes incompatible for ligation, and under such situation, LP-BER pathway is adopted [62]. In SP-BER, only one nucleotide is incorporated and the nick is sealed by DNA ligase III (LIG3) [11]. In LP-BER, a short patch of up to 6-9 nucleotides is incorporated [220], resulting in the displacement of the non-template DNA strand ahead of the synthesis. This creates a 'flap' like structure, which is processed by the structure-specific endonuclease flap endonuclease 1 (FEN1) [219, 273], assisted by the DNA replication ATP dependent helicase/nuclease 2 (DNA2) [221, 274], regenerating a ligatable 5′-phosphate end. Alternatively, EXOG (exo/endonuclease G) can also achieve this step [275]. Finally, LIG3 seals the nick [11]. It is interesting to note that various intrinsic and extrinsic insults instigate SSBs, which are also generated during the processing of damaged bases during BER. Hence, SSBs inflicted under diverse circumstances are processed and repaired by many of the same enzymes that participate during the later stages of BER [106].

Even though the majority of BER proteins are found in both the nuclear and mitochondrial compartments, a subset of proteins participating in BER selectively localize within mitochondria, thereby making mitochondrial BER highly efficient. In addition, alternative promoter usage and alternative splicing produce protein isoforms with selective sub-cellular localization, e.g., by encoding MTS and nuclear localization signal. Glycosylases heavily depend on such regulatory mechanisms to constitute organelle-specific pools [276]. APE1 [277, 278] and FEN1 [279] are other BER proteins with MTS encoded in their isoforms. Major examples of proteins with exclusive mitochondrial localization include the EXOG nuclease that removes flaps produced during LP-BER [275, 280] and pol γ that functions both in replication and repair activities within these organelles [207, 269, 281].

5.1.3 Nucleotide Excision Repair

NER, a highly versatile DNA repair pathway, deals with a wide variety of bulky lesions that provoke structural deformity to the DNA helix [106, 206]. Typical examples of such lesions include pyrimidine dimers such as CPDs and 6-4PPs generated by the UV component of sunlight [106]. Interestingly, cisplatin-intrastrand crosslinks also represent important substrates of NER. Even though mechanistically very similar to BER, NER is comparatively more complex that requires the participation of a large number of proteins to mediate a 'cut-and-patch' like mechanism [106]. The nuclear NER system is divided into two sub-pathways, viz., global genome NER and transcription-coupled NER (TC-NER). *In vitro* reconstitution experiments have revealed that mammalian nuclear NER requires the participation of at least 25 distinct proteins, including 7 factors [Xeroderma Pigmentosum Group A (XPA) to Xeroderma Pigmentosum Group G (XPG)] involved in the human disease Xeroderma Pigmentosum and two proteins [Cockayne syndrome Group A (CSA) and Cockayne syndrome Group B (CSB)] associated with Cockayne syndrome [282]. Briefly, NER commences with damage recognition followed by cleavage of the damaged strand at both sides flanking the lesion. Then, the missing sequence is synthesized using the complementary strand as a template. Finally, the ends are sealed, thereby restoring DNA sequence and integrity. A comprehensive review of nuclear NER can be found elsewhere [283].

Initial attempts to detect DNA repair in mitochondria examined elimination of UV-induced DNA damage, the prototypical NER substrate, from mtDNA and found that these lesions were not eliminated at significant rates from mammalian [202] or yeast cells [203], leading to the long-standing notion that mitochondria are devoid of any DNA repair activity. To date, there is no clear evidence of the occurrence of canonical NER in mitochondria

[11]. Even though a complete NER pathway is apparently absent in mitochondria, multiple nuclear NER factors have been shown to localize to mitochondria in mammalian cells. Initial evidence of a mitochondrial role for NER factors was from the observation that 8-oxoG elimination from mammalian mtDNA was impaired in cells deficient in TC-NER protein CSB, which is mutated in Cockayne syndrome [284]. Later, it was found that in cells with CSB deficiency, the activity of mitochondrial BER toward 8-oxoG, uracil, and 5-hydroxyuracil was decreased compared to wild-type cells. In that study, CSB was shown to be present in mitochondria of human cells, with the level of CSB increasing in mitochondria in response to oxidative stress [227]. A separate study demonstrated that CSB and CSA translocate into mitochondria only during oxidative stress, where they bind to mtDNA and a few proteins involved in BER (OGG1 and mtSSB), thereby promoting mtDNA repair [228]. The discrepancy of CSB localization to mitochondria under different conditions in two different studies could be due to different cell lines and techniques used to track CSB. Nonetheless, this behavior of CSB and CSA suggests their participation in mitochondrial response to oxidative stress [285]. It has also been revealed that both CSA and CSB protect mtDNA against common deletion [228]. Moreover, mitochondrial CSB has been shown to promote transcription elongation of mtDNA [286]. As nuclear NER involves multiple other enzymes that are not known to be present in mitochondria, it is plausible that either NER mechanism in mitochondria differs from the nuclear one or that CSB and CSA in mitochondria are repurposed to perform some other functions, e.g., take part in BER and transcription as they do in the nucleus. In the nucleus, these proteins participate in the transcription initiation, TC-NER, and BER [285].

Another known participant of NER, Xeroderma Pigmentosum Group D (XPD), which is a component of the basal transcription factor TFIIH, has been shown to localize to human mitochondria

[229]. Upon oxidative stress, XPD is actively recruited to mitochondria and XPD-deficient cells accumulate more mtDNA deletions following H_2O_2 treatment compared to XPD-proficient or complemented cells, implicating a role of mitochondrial XPD in the repair of oxidatively induced DNA damage. It is still unclear if this is through a role of XPD in mitochondrial BER; however, it should be noted that among cell lines deficient in the NER proteins XPA, XPB, and XPD, lymphoblastoid cells deficient in XPD are the most sensitive to H_2O_2 induced genome instability [287]. Even though many NER factors do not seem to play a role in mtDNA repair, NER-deficient cells display mitochondrial dysfunction, which may contribute to the pathophysiology of DNA repair disorders [288]. As mtDNA repair mechanisms have not been studied comprehensively, elucidation of the involvement of individual components in this process requires further investigation. The presence of NER in mitochondria has not been demonstrated, so it is most likely that this mechanism is not implemented in mitochondria in its classical form [285].

5.1.4 Mismatch Repair

In the nucleus, the MMR is an important post-replication repair mechanism dedicated to rectifying the misincorporation of bases that have eluded the proofreading activity of replication polymerases. Moreover, polymerase slippage during replication of repetitive DNA sequences is known to create insertion/deletion loops (IDLs), which are also effectively removed by proteins involved in MMR [106]. Accordingly, the MMR pathway contributes to the fidelity of replication as it targets the newly synthesized DNA strand for repair [289]. The MMR pathway can be broadly divided into three major steps: a recognition step in which mispaired bases are identified, an excision step in which the strand harboring the erroneous nucleotide is degraded resulting in a gap that is filled in the final repair-synthesis step. In

humans, the nuclear MMR pathway is driven by two major protein complexes, viz., MutS and MutL, based on their homology to the MMR proteins from *E. coli*. In essence, MutS is responsible for the initial mismatch recognition, while MutL links MutS-mediated recognition of mispaired bases to the downstream MMR events [106]. In mammals, two MutS complexes (MutSα and MutSβ) exist, each of which functions as a heterodimer (MSH2-MSH6 and MSH2-MSH3, respectively). The MutSα heterodimer preferentially recognizes base-base mismatches and IDLs of one or two nucleotides, whereas the MutSβ heterodimer detects larger IDLs [106]. After recognizing and binding to its substrate, MutS undergoes an ATP-driven conformational change and recruits MutL heterodimer, the major downstream MMR protein complex [289]. Among three MutL heterodimers (MutLα, MutLβ, and MutLγ) identified so far, MutLα possesses the primary MutL activity (~90%) that mediates repair initiated by both MutSα and MutSβ [106]. A comprehensive detail on nuclear MMR is reviewed in [290].

Even though the nuclear MMR pathway has been well characterized, not much is still known about mitochondrial MMR. The initial evidence that mitochondria possess MMR activities was found in yeast *S. cerevisiae*, in which MutS homolog (MSH1) localized to mitochondria [291]. MSH1 deficient yeast strains exhibited higher mtDNA mutations and large-scale rearrangements [292, 293], and strong instability in poly-GT tracts [294], suggesting MSH1 role in mtDNA stability. Purified yeast MSH1 binds to DNA substrates containing mismatches and unpaired nucleotides, having a substrate specificity comparable to that of bacterial MutS [295]. In this organism, MSH1 can repair G:A mispairs in mtDNA, which are produced by replication past 8-oxodeoxyguanosine (8-oxodG), as well as other mismatches [296, 297]. Apart from its role in mitochondrial MMR, MSH1

has been shown to be directly involved in the repair of oxidative damage in the mtDNA [293, 296] through a role in BER [298], and in large-scale recombination of the mtDNA [299]. In addition to MSH1, a MutL homolog (MLH1) has also been detected in *S. cerevisiae* mitochondria [206, 300]. *A. thaliana* also harbors a MutS homolog (AtMSH1) in its mitochondria [301]. The DNA-binding activity of AtMSH1 has been shown recently [302]. AtMSH1 has been implicated in mtDNA recombination [303] and maintenance of mtDNA copy number [304].

Evidence indicates that mammalian mitochondria may possess a novel MMR activity [217] that is most likely distinct from the nuclear counterpart [206]. The fact that mtDNA instability in mammalian cells has been rarely associated with defects in nuclear MMR genes [305] supports the notion that MMR machinery in the mitochondrial and nuclear compartments are independent of each other [62]. Mason et al. initially demonstrated that mammalian mitochondria were capable of MMR. They noticed that mitochondrial extracts from rat liver repaired G:T and G:G mismatches. However, they could not detect MSH2, a key component in nuclear MMR, in those extracts, indicating that mitochondrial MMR activity employs different components to those found in the nucleus [306]. One study did suggest that rat liver mitochondria may harbor MSH2 [307]; however, mitochondrial extracts from MSH2-deficient cells revealed similar mismatch binding activity [308], further confirming that mitochondrial MMR is independent of MSH2. Even though MutS homolog has not been identified in mammalian mitochondria, different studies have reported the presence of MLH1 in mitochondria of human tumor cells [309] and mouse liver cells [310]. Interestingly, data suggest a role for MLH1 in mitochondrial oxidative DNA repair. MLH1 deficiency together with silencing of the mitochondrial genes *POLG* and *PINK1*, among others causes an accumulation

in mitochondrial 8-oxoG lesions, which is incompatible with cell viability [309, 311]. Overexpression of MLH1 in retinal endothelial cells demonstrated that MLH1 has a protective effect in mtDNA following glucose-induced DNA damage [312]. Thus, MLH1 appears to play an important role in the maintenance of mtDNA.

The key factor involved in human mitochondrial MMR responsible for mismatch recognition and binding has been revealed to be Y-box binding protein 1 (YB-1) [308]. This mammalian mitochondrial protein detects and binds the mismatched DNA and small IDLs, denaturing the mtDNA strands, and initiating repair [308]. The involvement of YB-1 in mitochondrial MMR is underscored from the observation that soluble proteins from YB-1-depleted mitochondrial extracts interact less efficiently with mismatches and IDLs. These YB-1-depleted mitochondrial extracts also show lower mismatch correction activity *in vitro* that is complemented by recombinant YB-1 [11, 308]. Moreover, silencing of YB-1 is known to evoke mtDNA mutagenesis [198, 308]. These observations support the idea that YB-1 participates in the mitochondrial MMR pathway [10]. YB-1, after performing its function, is thought to recruit the downstream mitochondrial MMR complex, the identity of which is currently unknown [62]. Thus, functionally, YB-1 is thought to be analogous to nuclear mismatch recognition complexes MutSα and MutSβ [308]. Taken together, the mammalian mitochondrial MMR pathway is far from being fully characterized as additional factors involved in the process are yet to be identified [217].

Even though proteins involved in MMR have been found to localize in mitochondria, it is still debated if mitochondria possess efficient MMR activity *in vivo*. A little evidence for mitochondrial MMR activity has been reported; however, it remains to be demonstrated if this repair pathway functions within mitochondria, as these organelles seem to lack key proteins that perform nuclear

MMR, for example, MSH2, MSH3, and MSH6 [306, 308]. So far YB-1 is the only protein that has been shown to directly participate in human mitochondrial MMR. Interestingly, YB-1 also interacts with the glycosylase NEIL2 and the nuclease APE1, indicating the functional crosstalk between MMR and BER may be present for the maintenance of mtDNA [308].

As noted earlier, proteins from one repair pathway can function in another repair pathway. So, it may be the case that proteins from other repair pathways, like BER, may participate in repairing mitochondrial mismatches and have roles in downstream events of the mitochondrial MMR pathway. Future studies will be required to reveal if that is indeed the case.

5.1.5 Double-Strand Break Repair

In the nucleus, DSBs are considered the most detrimental form of DNA damage, repair of which is crucial not only for the maintenance of genome integrity and cellular homeostasis but also for cell viability [106, 313]. These lesions can occur spontaneously as a result of replication stalling, ROS-induced damage, or ionizing radiation. Even though it is not always clear what triggers spontaneous recombination events, but they can be stimulated by DSBs [10]. In the nucleus, there are two predominant pathways to tackle these lesions: homologous recombination (HR) (reviewed in [314]) and non-homologous end joining (NHEJ). These pathways differ in their requirement for a homologous DNA template as well as in the fidelity of repair. HR, as its name suggests, repairs DSBs by utilizing the undamaged sister chromatid as a template to restore the lost genetic information, and for this reason, it is, by and large, an error-free mechanism. On the other hand, NHEJ eliminates DSBs by direct ligation of broken ends [106]. One of the most notable aspects of NHEJ is the diversity of substrates that it can use and convert them to ligatable products [315]. This

pathway does not require a homologous sister chromatid to initiate repair, so it is not restricted to any particular phase of the cell cycle, whereas HR is largely constrained to the late S and G2 phases when the homologous DNA template is available in the form of a sister chromatid [106, 316]. Therefore, the choice of these pathways depends largely on the phase of the cell cycle [11]. NHEJ repair pathways are categorized into well-defined classical NHEJ (C-NHEJ) pathway (reviewed in [315]) and comparatively less characterized alternative NHEJ (A-NHEJ) pathway, also referred to as microhomology-mediated end joining (MMEJ) (reviewed in [317]). During C-NHEJ, minimal end processing occurs that is followed by ligation of the processed ends. The end processing frequently results in small deletions or insertions at the DSB site thereby making C-NHEJ an error-prone process. MMEJ is a subpathway of NHEJ that is believed to function in the absence of canonical NHEJ factors and utilizes microhomologies, generally 5-25 nucleotides, which are revealed during end processing to facilitate the ligation of the broken ends. Because MMEJ results in the formation of short flaps that are removed before end-joining, this pathway frequently results in mutational events, mainly small-scale deletions, though these deletions are larger than those seen in C-NHEJ [10, 207, 317, 318].

5.1.5.1 Double-Strand Break Repair in Mammalian Mitochondria

The study of mtDNA DSBR is an area of active research. Initial studies utilized constitutive expression of mitochondrial-targeted restriction endonuclease *Pst*I (mito*Pst*I) in skeletal muscle of mice [319]. As mouse mtDNA contains two *Pst*I sites, transgenic founders displayed a mitochondrial myopathy associated with mtDNA depletion [319]. The residual level of wild-type mtDNA in muscle of the founders was ~40% of controls [319], suggesting

that the continual cleavage of mtDNA likely overwhelmed the mouse mtDNA DSBR system leading to mtDNA depletion. The study identified the formation of large mtDNA deletions in muscle of transgenic mice. A family of mtDNA deletions was detected, and most of these rearrangements involved one of the *Pst*I sites and the 3′ end of the D-loop region. The deletions had small or no direct repeats at the breakpoint region. These characteristics were identical to the ones seen in humans with multiple mtDNA deletions in muscle, indicating that DSBs mediate the formation of large mtDNA deletions [319]. To study the effects of more transient DSBs on mouse mtDNA, a subsequent study utilized a neuronal-specific tetracycline-regulated mito*Pst*I inducible system [320]. These mice did not show any significant reduction in mtDNA levels following transient expression of mito*Pst*I [320], indicating that if mtDNA DSB levels are lower than a threshold, cells can efficiently repair them. The researchers were able to detect mtDNA deletions in mice that had undergone DSB induction and such deletions were absent in control mice [320]. The study identified transient induction of mtDNA DSBs led to the formation of a family of partially deleted mtDNA with characteristics that closely resembled naturally occurring mtDNA deletions. The researchers hypothesized that the formation of the deleted species was likely mediated by yet uncharacterized DSBR machineries. Moreover, the study demonstrated that mtDNAs with larger deletions accumulated faster than those with smaller deletions, indicating a replicative advantage of smaller mtDNAs. Thus, the study concluded that DSB, DSBR systems, and replicative advantage as likely mechanisms underlying the formation and age-associated accumulation of partially deleted mtDNA in mammalian neurons [320]. The description of mechanisms that are potentially involved in repairing mtDNA DSBs in mammalian mitochondria are provided ahead.

5.1.5.1.1 Homologous Recombination

In the nucleus, HR is limited to the late S and G_2 phases of the cell cycle, when a fully copied sister chromatid is available. This limitation of nuclear HR would not exist in mitochondria, as multiple copies of mtDNA are generally present in a mitochondrion at any given time [11]. In this context, many nuclear HR proteins have been identified in the mitochondrial compartment. For example, Rad51, a key component of nuclear HR, has been detected in mitochondria of human cells where it was shown to bind to mtDNA following exposure of cells to oxidative stress [321]. In the same study, it was also demonstrated that paralogs of Rad51, viz., Rad51C and XRCC3, also localize to mitochondria of human cells, and contents of all these three proteins in mitochondria were increased after induction of oxidative stress. Moreover, depletion of any of these factors caused a dramatic decline in mtDNA copy number [321], implying some form of HR involvement in mitochondrial genome maintenance. Interestingly, Rad51 recombinase enters mitochondria in response to induced oxidative stress and this translocation necessitates an active mtDNA replication. Upon inhibition of mtDNA replication, the phenomenon was not detected i.e., Rad51 was not found in mitochondria even under the oxidative stress condition [322]. In addition to Rad51 and related proteins, Mre11, a component of the MRN (Mre11, Rad50, Nbs1) complex, has been identified in mammalian mitochondria bound to mtDNA [323]. Recently, other MRN complex proteins have also been shown to be present in mitochondria [324]. In the nucleus, the MRN-CtIP complex is involved in the initial DSB recognition and end-resection priming step of HR. This complex also participates in MMEJ [11, 325]. The MRN complex interacts with BRCA1 to promote end resection in nuclear HR. BRCA1 has been shown to be present in mitochondria, where it colocalizes with mt-nucleoids, possibly functioning in HR-mediated DSBR

[326]. Another important component identified in mitochondria that may potentially participate in mitochondrial HR is DNA2 [221]. EXOG, a mitochondrial 5′-exo/endonuclease, is hypothesized to be the mitochondrial equivalent of nuclear Exo1 that may be involved in the production of 3′-ssDNA tails. Mitochondrial proteins TFAM and Twinkle are also involved in mtDNA recombination in mammals as the deficiency of these proteins causes increased content of unresolved Holliday structures in mtDNA [327]. MtSSB and mitochondrial genome maintenance exonuclease 1 (MGME1), the latter can act as an exonuclease, are also present in mammalian mitochondria [328]. Apart from resolvase and Rad52-like protein, mammalian mitochondria appear to harbor the basic apparatus of HR [285] (Table 2). Based on these observations, it is plausible to suggest that some form of recombination-based process can occur in mammalian mitochondria.

Early reports about mitochondrial HR revealed that mitochondrial protein extracts from mammalian cells can catalyze HR of plasmid DNA substrates, suggesting that mitochondria harbor HR machinery to perform this activity [329]. Moreover, following preincubation of mitochondrial protein extracts with anti-RecA antibodies, inhibition of HR reaction was noticed, indicating that a homolog of the bacterial strand-transferase protein RecA (viz., Rad51) is involved in HR activity in mammalian mitochondria [329]. Another study demonstrated that HR is an important DSBR mechanism in mitochondria, where it functions in the maintenance of the mtDNA integrity [324]. The study revealed that mitochondrial extracts from various rat tissues could successfully perform HR-mediated repair *in vitro*. Biochemical studies showed that HR-mediated repair of DSBs was more efficient in the mitochondria of testes as compared to that of brain, kidney, and spleen. Interestingly, induction of a DSB significantly increased the efficiency of HR. The study showed the presence

of Rad51 and MRN complex proteins in the mitochondria. Moreover, immunodepletion of Rad51, Mre11, or Nibrin (Nbs1) suppressed the HR-mediated repair [324]. Intriguingly, findings of a recent study suggest that mitochondria also utilize HR to repair endogenous and xenobiotic-induced DNA-protein crosslinks [330]. The study revealed a higher accumulation of cisplatin-induced mtDNA-protein crosslinks in cells pretreated with the Rad51 inhibitor B02 compared to control cisplatin-treated cells [330].

It has been demonstrated that HR is indispensable for the preservation of mtDNA in plants, yeast, and fungi [331]. Indeed, the phenomenon of HR has been well documented in the mitochondria of *S. cerevisiae*. Due to the biparental inheritance of yeast mtDNA, HR in this organism is easy to detect [332, 333]. In mammalian systems, however, detection of recombinant mtDNA molecules is difficult because mtDNA is maternally inherited and recombination events between the homologous molecules would produce indistinguishable copies of mtDNA [11]. A study utilized inducible expression of mitochondrial-targeted restriction endonuclease *Sca*I in heteroplasmic cells and heteroplasmic mice to generate DSBs in mtDNA [334]. After transient expression of the restriction endonuclease followed by a long period of recovery, both intramolecular and rare intermolecular recombination products containing large deletions were identified, indicating that induction of DSBs in mtDNA promotes recombination resulting in large deletions. The recombination event occurred more frequently between molecules of the same haplotype, even though DNA exchange between different haplotypes also occurred [334]. This suggested that recombination between mouse mtDNA can occur, at least under such experimental conditions.

Even though there exists evidence in favor of HR in mammalian mitochondria, its significance *in vivo* is still vague. So far there is no solid proof for the presence of HR in mammalian mitochondria; however, experimental data published suggest that mammalian mitochondria possess a DNA repair mechanism that closely resembles HR [285]. Human and mammalian mtDNA have shown the presence of circular dimers and catenanes [72]. Circular dimers are especially numerous in human leukocytes in leukemia and in some cultured cell lines. It is postulated that such structures are formed as a result of HR [285]. Moreover, mtDNA in the adult human heart is organized in a network of large molecules containing dozen of genomes. These interlaced genomes contain classical Holliday junctions (branched structures and abundant four-way junctions) generated most likely as a result of HR. Other human tissues do not possess such type of mtDNA structures, except for the brain, in which the network is thinner, but mtDNA molecules are intertwined [75]. As the adult human heart and brain contain the most functionally active mitochondria, it may be the case that active HR occurs in tissues with high energy demand. *In vivo* relevance of HR was also seen in patients who inherited both maternal and paternal mtDNA, in which recombinant mtDNA molecules were easily detected [335, 336].

Our understanding of the factors involved in mitochondrial HR has increased over the past few years. Evidence is also accumulating in favor of the existence of mitochondrial HR in mammals. However, our current knowledge of the exact roles of potential participating proteins and the underlying molecular mechanisms of how HR operates in mitochondria is still lacking compared to the nuclear models.

5.1.5.1.2 Classical Non-Homologous End Joining

The nuclear C-NHEJ begins with DNA end-binding by Ku heterodimer, Ku70/Ku80, which is believed to protect DNA ends

and keep them in close proximity for ligation. Integral to the C-NHEJ pathway, this complex detects and binds DSB ends and recruits other repair proteins to the damaged sites [337]. To my knowledge, there are only two studies indicating that C-NHEJ may operate in mammalian mitochondria. The first study investigated the ability of mammalian mitochondrial protein extracts to catalyze the repair of DNA DSBs introduced into plasmid DNA via restriction digestion following which repair products were analyzed [338]. End joining was reported to be highly precise in the case of linearized DNA containing cohesive ends whereas DNA containing blunt ends were rejoined with decreased precision and efficiency. Molecular characterization of all imprecisely repaired products showed DNA deletions spanning two direct repeats (which were likely formed by MMEJ). As the deletions observed in the study were very similar to those present in mitochondrial genomes of aged humans and patients with certain mitochondrial diseases (e.g., Kearns-Sayre syndrome, Pearson syndrome, etc.), the study concluded that mammalian mitochondria can repair DSBs and the repair pathway(s) likely play(s) a role in generating mtDNA deletions seen in a number of human pathologies [338]. The second study revealed that mitochondrial protein extracts prepared from a hamster cell line lacking Ku80 mRNA expression were devoid of the DNA end-binding activity, which was present in similar extracts prepared from wild-type cells [339]. Immunoblotting of mammalian mitochondrial protein extracts with a monoclonal antibody specific for an N-terminal epitope of Ku80 detected a 68 kDa protein. However, this mitochondrial protein could not be detected by a monoclonal antibody specific for a C-terminal epitope of Ku80. The study concluded that a C-terminally truncated form of Ku80 is present in mammalian mitochondria that performs DNA end-binding activity and that Ku80 gene expression is necessary for mammalian mtDNA end-binding [339].

Even though mitochondrial localization of Ku70 has not been reported, the stability of each Ku subunit depends on the other, and knockout of either or both displays a similar phenotype in mice [340], implying that both subunits would likely be required for mitochondrial C-NHEJ, should it occur. In the nucleus, following Ku binding to DNA ends, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which along with Ku forms the full DNA-dependent protein kinase (DNA-PK), Artemis, a nuclease the activity of which is regulated by DNA-PKcs-mediated phosphorylation, and DNA ligase IV, are recruited to repair the breaks [315, 341]. These downstream C-NHEJ proteins, except DNA-PKcs [342], have not been detected in mammalian mitochondria to date. Moreover, mammalian mitochondrial extracts from different tissues do not harbor the machinery to repair DSBs via C-NHEJ *in vitro* at the same concentrations at which whole-cell extracts can perform proficiently [342]. Therefore, it is still unclear whether C-NHEJ operates in mitochondria and if it is relevant for mtDNA maintenance [11].

5.1.5.1.3 Microhomology-Mediated End Joining

In contrast to C-NHEJ, evidence is accumulating for the presence of MMEJ in mammalian mitochondria. This pathway uses microhomologous sequences, generally ranging from 5-25 nucleotides in length, to repair DSBs and is independent of C-NHEJ core proteins like Ku70/Ku80 [62, 317]. In nuclear MMEJ, poly-ADP-ribose polymerase (PARP1) acts as the DSB sensor, competing with Ku70/Ku80 to occupy and repair DSBs via the two distinct pathways [343, 344]. It has been shown that PARP1 localizes within mitochondria, associating with mtDNA and participating in mtDNA maintenance [345]. In nuclear MMEJ, PARP1 is involved not only in DSB recognition but also in the recruitment of the MRN-CtIP complex to the site of DSB [325]. Mre11, which forms a core of the MRN complex, has

been found in mammalian mitochondria, colocalizing with and binding to mtDNA [323]. The FEN1 flap endonuclease localizes to mitochondria, where it participates in mitochondrial LP-BER [219], is also implicated in MMEJ, most likely by cleaving the 5′ flap at DSBs [346]. In the nucleus, the DNA ligase that performs the ligation step in MMEJ is LIG3, which is also present in the mitochondria as the only ligase [11]. The presence of different MMEJ factors in the mitochondria implies that this pathway, which competes with C-NHEJ in the repair of DSBs, likely operates within these organelles. In fact, a study has revealed that mitochondrial extracts from various rat tissues and HeLa cells can efficiently repair DSBs through MMEJ but not through C-NHEJ [342]. In the study, mitochondrial and nuclear-cytoplasmic extracts were mixed with different C-NHEJ substrates. In the mitochondrial extracts, DNA repair did not take place with any of the substrates tested, whereas end-joining occurred for all the substrates used in the nuclear-cytoplasmic fractions. These results suggested the absence of C-NHEJ in the mitochondria. To see the presence of MMEJ, the same extracts were mixed with oligonucleotide pairs with 13-nucleotide microhomologous direct repeats at their ends. The researchers observed both C-NHEJ and MMEJ activity in the nuclear-cytoplasmic extracts whereas only MMEJ activity was present in the mitochondrial extracts [342]. Thus, these results demonstrated the existence of MMEJ, but not C-NHEJ, in mammalian mitochondria.

The study performed by Tadi et al. [342] revealed that mammalian mitochondrial extracts could join oligomeric dsDNA molecules containing direct repeats that differ in length. The study reported that a minimum of 5-nucleotide microhomology was essential for efficient MMEJ in mitochondria. Robust MMEJ occurred with DNA substrates bearing 5-, 8-, 10-, 13-, 16-, 19-, and 22-nucleotide microhomology, and the efficiency of joining

was further enhanced as the length of the microhomology was increased. To reveal factors involved in mitochondrial MMEJ, the mitochondrial extracts were depleted immunologically using antibodies against proteins involved in C-NHEJ and MMEJ. While removal of Ku70, Ku80, or DNA-PKcs did not affect the efficiency of MMEJ, removal of CtIP, FEN1, LIG3, Mre11, and PARP1 significantly decreased microhomology-mediated joining. Therefore, it was concluded that *in vitro* mitochondrial MMEJ activity depends on known nuclear MMEJ factors, and mtDNA DSBR via MMEJ occurs with the participation of CtIP, FEN1, LIG3, Mre11, and PARP1 [342].

It is important to note that the MMEJ mechanism results in the deletion of one of the direct repeats. In the study performed by Tadi et al., the observed deletions at the MMEJ junctions and efficient use of microhomology regions by mitochondrial proteins displayed a striking resemblance to deletions observed within the mtDNA of patients with mitochondrial dysfunction [342]. Interestingly, more than 100 different types of mtDNA deletions have been reported, of which ≥85% are flanked by short direct repeats and are implicated in cancer, aging, mitochondrial syndromes, and other diseases [347-355]. One of the hallmarks of the observed mitochondrial genome rearrangement is the retention of one of the repeats after breakage and rejoining. Therefore, the characteristic features of MMEJ in mitochondria, as reported by Tadi et al., are comparable to those observed during mtDNA deletions in patient samples [342]. Collectively, the MMEJ observed in the study provides a plausible mechanism for the production of mtDNA deletions seen in specific mitochondrial disorders.

A large number of mtDNA deletions noticed *in vivo* span direct repeat sequences of 5-13 bp in length [356, 357]. The study performed by Tadi et al. revealed that a minimum of 5-nucleotide microhomology was required for efficient MMEJ in mitochondria.

Furthermore, the efficiency of joining was enhanced when the length of the microhomology was increased, which correlates with the *in vivo* data [342]. The mechanism of this pathway also explains the common deletion observed in the human mitochondrial genome that occurs between two 13 bp direct repeats located ~5 kb apart [62]. Additionally, the results from the study performed by Tadi et al. are reinforced by a previous study utilizing mito*Pst*I restriction endonuclease to instigate DSBs in mice mtDNA, in which the repaired mtDNA exhibited small direct repeats at the breakpoint region [319].

It is still an open question if efficient DSBR is present in mammalian mitochondria. MtDNA molecules containing DSBs in mammalian cells are quickly degraded by a process requiring the replication proteins pol γ, Twinkle, MGME1, and nucleoid/transcription protein TFAM [109, 207, 210, 211, 358]. This implies that DSBs in mtDNA are handled differently than the repair-oriented mechanisms occurring in the nucleus [207] . Even though experiments performed *in vitro* with mitochondrial extracts and isolated mitochondria, and *in vivo* with cell cultures and animal models indicate that both homology-dependent and -independent repair of DSBs may operate in mitochondria, more studies are necessary to fully demonstrate the underlying mechanisms and factors involved.

5.1.5.2 Double-Strand Break Repair in Yeast Mitochondria

The molecular mechanisms of HR have been thoroughly described in the nucleus, including the major participating enzymes such as exonuclease that produces the free 3′-ends, the ssDNA-binding protein that maintains these ends in the unpaired state, the Rad52-like protein that interacts with the free ends and attracts Rad51 recombinase, and resolvase that resolves Holliday junctions [359]. Helicases that unwind dsDNA and ligases that join nicks

also participate in the recombination process. Yeast mitochondria, which are renowned to be a site of active recombination, contain all enzymes necessary for this process, including mitochondrial resolvase [285, 360, 361] (Table 2).

Table 2 Main enzymes of HR in yeast and human mitochondria. This table is adapted from [285].

Proposed function in homologous recombination	Organism (mitochondria)	
	S. cerevisiae	*Homo sapiens*
Helicase	Pif1	Twinkle
Exonuclease	MRX	MRN/MGME1
Single-stranded DNA-binding protein	Rim1	mtSSB
Rad52-like protein	Mgm101	?
Recombinase	Mhr1/Rad51	Rad51
DNA-binding structural protein	Abf2	TFAM
Resolvase	Cce1	?

Note: ?, no protein with such function has been determined.

Recombination of the mitochondrial genome is well accepted in yeast [62, 204]. A variety of proteins potentially involved in recombination have been identified in yeast mitochondria. These include endo/exonucleases such as Nuc1, Din7, Exo5, Rad27, and MRX (Mre11, Rad50, Xrs2; MRN in mammals), which can perform the initial priming and resection of DSBs [11, 285, 359, 362]. Mgm101, a Rad52-like protein, is another factor required for homologous recombination of yeast mtDNA [363]. Mhr1 functions in yeast mtDNA replication and performs an essential role in the pairing of homologous strands for recombination [33]. Other

proteins that may participate in mtDNA recombination include mtSSB protein Rim1, 5′→3′ DNA helicase Pif1, Rad51, and Rad59 [206, 285, 364]. Finally, a Holliday junction endonuclease, Cce1, is also present in yeast mitochondria, where it functions as the resolvase, thereby completing the last enzymatic step in the HR pathway [365, 366]. Mre11 is present in the proteome of yeast mitochondria [300] and has also been detected in mammalian mitochondria bound to the mtDNA [323]. A study has revealed that yeasts devoid of the MRX complex have mtDNA rearrangements, indicating that the complex participates in mitochondrial as well as nuclear DSBR of lower eukaryotes [367]. It has been reported that loss of the MRX and Ku70/80 complexes substantially impacts the rate of spontaneous deletion events in mtDNA, and that these proteins contribute to the repair of induced mtDNA DSBs [367]. Moreover, the data of the study supported the notion that HR is the predominant pathway by which mtDNA deletions arise in yeast [367]. A separate study demonstrated that Rad51 and Rad59, proteins involved in nuclear HR, localize to budding yeast mitochondrial matrix and that Rad51 physically interacts with the yeast mitochondrial genome [364]. By utilizing a mitochondrial-targeted restriction endonuclease *Kpn*I to introduce a unique DSB in the mitochondrial genome, the study revealed that repair of induced mtDNA DSBs was impaired as a result of loss of Rad51 and Rad59 [364]. Furthermore, the loss of Rad51, Rad52, and Rad59 significantly decreased the rate of spontaneous mtDNA deletion events [364]. These observations suggest that these nuclear HR proteins likely participate in the repair of mtDNA DSBs and that the repair process may be involved in the generation of mtDNA deletions [364].

A study in *S. cerevisiae* demonstrated that Mhr1 could bind to restriction endonuclease *Xho*I-induced mtDNA DSBs *in vivo*, suggesting its involvement in mtDNA DSB repair [368]. This

property of Mhr1, along with its homologous pairing activity *in vitro* [33], supports the postulation that Mhr1 is a general mtDNA DSB repair factor in *S. cerevisiae* [368]. The study also showed that Ku80 does not compete with Mhr1 for binding to mtDNA DSBs and, in fact, C-terminally tagged Ku80 could not be detected in yeast mitochondrial extracts. The study concluded that Mhr1, but not Ku80, is a potential mtDNA DSB repair factor in yeast and proposed that Mhr1-mediated HR is probably the main pathway by which yeast cells repair mtDNA DSBs [368].

Interestingly, recombination is also an important mechanism for the propagation of the mitochondrial genome in *S. cerevisiae*. As recombination initiates following a DSB, this lesion, apparently, is not always deleterious, at least in the case of *S. cerevisiae* mitochondrial genome. Indeed, different studies have shown that DSBs can mediate mtDNA replication in respiratory-competent as well as respiratory-deficient yeast cells [369-371]. Therefore, controlled damage of mtDNA seems to be an important strategy adopted by yeast cells to maintain their mitochondrial genome. It is also likely that other organisms adopt such strategy to propagate their mtDNA.

6

MITOCHONDRIAL DNA DEGRADATION

In the nucleus, pyrimidine dimers are removed by the NER pathway. As initial experiments failed to show evidence of mtDNA repair of pyrimidine dimers [202, 372], it was hypothesized that mtDNA was not repaired and instead damaged genomes are simply degraded. This proposition was further reinforced after a demonstration that mitochondrially-localized endonuclease G preferentially cleaved mtDNA opposite SSBs produced after oxidative damage [373]. Recent studies have provided more direct evidence that selective degradation of mtDNA may act as a quality control mechanism in the mitochondria that removes heavily damaged mitochondrial genomes from the replication cycle [109, 148, 204, 209, 211, 319, 334, 358, 374-380].

Mitochondria, unlike the nucleus, possess an option to selectively degrade damaged mtDNA that is beyond the scope of repair [62, 217]. Since a typical cell contains thousands of copies of mtDNA [381], the degradation of severely damaged mtDNA molecules can be tolerated without compromising mitochondrial function [62, 204]. It has been proposed that mtDNA degradation acts as a protective mechanism to lower mutagenesis and to reduce the energy cost of repair [382]. Initial report implying the

presence of degradation of damaged mtDNA demonstrated that fragmented mtDNA contained a 15-fold higher level of 8-oxoG compared to intact circular mtDNA in rat liver mitochondria, indicating the existence of "an efficient repair or degrading system" [383]. The idea that mtDNA degradation is an important response to cellular stress is also supported by data obtained from cell cultures and animal models. In mouse embryonic fibroblasts, mtDNA loss and persistent mtDNA lesions were documented upon H_2O_2 treatment [384]. A separate study revealed a rapid decrease in the amount of mtDNA following treatment of rat hepatocytes with 2′,3′-dideoxycytidine or ethidium bromide [385]. Similarly, in mice, mtDNA degradation in various tissues, including liver, heart, skeletal muscles, and brain was observed following oxidative stress induction with ethanol administration [377, 378]. Depletion of mtDNA content was also noticed after cerebral ischemia-reperfusion injury in a rat model. This was followed by the restoration of mtDNA to a normal level 24 hours after reperfusion [375]. When experimental animals were treated with environmental carcinogens [386] or when the expression of mitochondrial BER enzymes was suppressed [387], mtDNA mutation load did not increase, indicating the presence of specific mechanisms to remove damaged mtDNA molecules [109]. For mtDNA maintenance and stress response, mtDNA degradation has emerged as an important mechanism [109]. This mechanism is believed to be nonspecific with respect to the type of DNA lesion and is triggered in response to difficult-to-repair DNA lesions or excessive mtDNA damage [210, 388].

In 2009, it was demonstrated that oxidative stress could elicit mtDNA degradation and that accumulation of linear mtDNA molecules occurred before degradation [148]. The degradation of mtDNA further increased when the BER pathway was inhibited, indicating a competition between repair and selective degradation

of mtDNA under conditions of oxidative stress [148]. Following induction of mitochondrial-specific DNA SSBs and AP sites, it was verified that mtDNA degradation is a direct and immediate consequence of mtDNA damage [389]. The rate of degradation differed based on the type of mtDNA damage [389] and cell type [388]. Subsequently, it was demonstrated that BER and mtDNA degradation are two main pathways to counteract mitochondrial AP sites, and that translesion bypass of AP sites is observed much less frequently [390]. Studies have shown that different proteins negatively regulate mitochondrial BER, among which TFAM binds to the damaged mtDNA in close proximity to an AP site. TFAM then inhibits mitochondrial BER activation by steric exclusion of BER proteins, such as glycosylases and the APE1 nuclease, thereby enhancing the degradation of the targeted mtDNA molecule [208, 209].

By targeting site-specific restriction endonucleases into mitochondria of mammalian cells, several studies have revealed that extensive or persistent DSBs lead to mtDNA degradation [210, 211, 319, 320, 334, 358, 374, 391], whereas low levels of DSBs result in repair [334]. In the yeast system, however, there was no detectable depletion of mtDNA following persistent DSB induction by different mitochondrial-targeted restriction endonucleases [10, 364, 367, 368]. This may have resulted from an enhanced capacity for DSBR in yeast, as HR in yeast mitochondria may be much more robust than in the mammalian counterparts [10].

The signal that triggers mtDNA degradation is assumed to be produced by stalled replication or transcription machinery on the damaged mtDNA template [62, 217]. As strand breaks and AP sites lack coding information, selective degradation of severely damaged mtDNA would play a crucial role in preventing mutagenesis and preserving the integrity of the mitochondrial genome [217].

Recent studies have identified protein components involved in degrading damaged mtDNA molecules. These include important factors of the mtDNA replication machinery, such as pol γ, Twinkle, and MGME1, and the DNA packaging nucleoid protein TFAM [109].

The human pol γ holoenzyme comprises a catalytic subunit and a dimeric form of its accessory subunit [392]. The catalytic subunit is made up of an N-terminal exonuclease domain, a connecting linker region, and a C-terminal polymerase domain. The catalytic subunit possesses multiple enzymatic activities, which include DNA polymerase activity, 3′→5′ exonuclease activity, and 5′-dRP lyase activity. The exonuclease activity cleaves ssDNA, making it a suitable candidate for digesting linearized mtDNA fragments [109]. The accessory subunit enhances DNA binding and promotes processive DNA synthesis [393].

Another component of the mammalian mtDNA replisome is the mtDNA replicative helicase, Twinkle [394]. Indispensable for embryonic development in mammalian systems, Twinkle is known to unwind mtDNA for mtDNA synthesis by pol γ [395]. Human Twinkle oligomerizes to form a hexamer and displays 5′→3′ helicase activity due to the conserved superfamily 4 helicase domain situated at its C-terminus [396]. Apart from Twinkle, other DNA helicases are also transported into the mitochondrion to function in mtDNA replication and repair. These helicases include RECQ4, DNA2, PIF1, and SUV3. Many of these DNA helicases are also involved in nDNA replication and repair, characteristics in contrast to Twinkle, which is known to function exclusively in the mitochondrion [394].

Like Twinkle, MGME1 also localizes exclusively to mitochondria [109]. MGME1, a 5′→3′ exonuclease, is believed to be a part of the mitochondrial replisome since it interacts with all three core components (pol γ, Twinkle, and mtSSB) of the minimal

mitochondrial replisome [397-399]. Therefore, it is not surprising that MGME1 plays a crucial role in the replication of mtDNA [400]. The accumulation of mtDNA replication intermediates in HeLa cells treated with MGME1 small interfering RNA (siRNA) indeed supports a role for MGME1 in the maintenance of mtDNA replication *in vivo* [400]. MGME1 cuts DNA, but not RNA or DNA-RNA hybrids, requires free 5′-ends to exert its function, and prefers ssDNA over dsDNA *in vitro* [328].

Human mtDNA replication is also dependent on TFAM since nascent transcripts formed from LSP are used in priming DNA synthesis [91]. TFAM possesses two HMG box domains that incorporate into the DNA minor groove on the LSP, HSP1, or nonspecific regions of the mtDNA [88, 89, 401]. TFAM binding distorts the mtDNA resulting in DNA bending [85, 86, 89, 401, 402]. This distortion allows the specific binding of POLRMT to the start site, where TFAM interacts with the N-terminus of POLRMT to recruit TFB2M (transcription factor B2, mitochondrial) that enables productive transcription initiation [403-407]. In addition to its role on mtDNA transcription and replication, TFAM packages mtDNA into nucleoids by imposing a sharp U-turn on mtDNA [88, 89] and cross-strand interactions [85]. TFAM knockout mice display embryonic lethality, implicating a crucial role of this protein in mtDNA maintenance [109].

Studies from different laboratories have suggested the participation of the exonuclease activity of pol γ in mtDNA degradation [211, 358]. A recent study utilized mitochondrial-targeted restriction endonucleases to instigate mtDNA DSBs giving rise to linear mtDNA molecules that were eliminated within hours by exonucleolytic activities [211]. In the study, HEK-293 cells capable of expressing mitochondrial-targeted restriction endonucleases were generated. Following mtDNA DSB induction by restriction cleavage, progressive degradation

of mtDNA occurred to produce a mixture of DNA fragments that varied from a few hundred to several thousand bp. MtDNA degradation occurred from both the 3′-end and 5′-end, implying the involvement of two different types of exonucleolytic activities. Given the familiar specificities of mitochondrial nucleases pol γ and MGME1, the authors verified the role of these enzymes in the degradation process by producing cells defective in pol γ 3′→5′ exonuclease activity and MGME1-null cells. Elimination of the 3′→5′ exonuclease activity of pol γ or inactivation of the mitochondrial 5′→3′ exonuclease MGME1 led to the severe hindrance of mtDNA degradation. Similar effects were not observed when inactivating other known mitochondrial nucleases including EXOG, ENDOG, APEX2, FEN1, DNA2, MRE11, or RBBP8. The study confirmed that the exonuclease activities of pol γ and MGME1 are necessary for the removal of linear mtDNA [211]. The importance of Twinkle helicase in mtDNA degradation was supported by the observation that siRNA knockdown of this protein's mRNA resulted in the accumulation of linear mtDNA fragments [211]. Thus, in cellular models harboring restriction endonuclease-instigated mtDNA DSBs, 3′→5′ exonuclease activity of pol γ, MGME1, and Twinkle helicase work together to remove linear mtDNA molecules. The study concluded that the components of mtDNA replication machinery were responsible for rapid degradation of linearized mtDNA molecules, thereby underscoring novel roles for the participating enzymes pol γ, MGME1, and Twinkle [211].

A separate study utilized mtDNA mutator mice and the derived lung fibroblasts harboring an exonuclease-deficient pol γ to elucidate the role of exonuclease activity of pol γ in eliminating the fragmented mtDNA molecules upon DSBs instigated by mitochondrial-targeted restriction endonucleases [358]. In both the liver of the mutator mice and cultured lung fibroblasts,

prolonged existence of the mtDNA fragments was noticed, which led to increased levels of mtDNA deletions. MtDNA degradation was independent of the DNA polymerase activity of pol γ or the origin of replication, indicating that a different population of pol γ molecules attach at the free dsDNA ends [358].

At present, the answer as to how the degradation mode of replication machinery is regulated is still vague. The high-resolution crystal structures of apo pol γ and the pol γ ternary complex have not cast light on the exonuclease mode of pol γ, or how the degradation mode is triggered [109]. It may be the case that pol γ adopts a different conformation in the degradative mode, and thus cooperates with Twinkle and mtSSB to execute such functions [408]. The level of dNTPs may well be another potential factor regulating the pol γ degradative mode, as documented in the case of T4 DNA polymerase [409]. Moreover, a transition between DNA synthesis and degradation by pol γ may rely on the homeostatic functions of autophagy, as has been documented in yeast [410]. Further studies are required to address these questions.

Recently, TFAM has also been proposed to facilitate the degradation of damaged mtDNA containing AP sites [209]. Among all types of DNA lesions known, AP sites, resulting from BER and spontaneous base loss, are the most prevalent type of endogenous DNA damage in cells. Existing at a steady-state level of ~30,000 AP lesions per cell [411, 412], AP sites can number in the hundreds in the mitochondria of each cell [413, 414]. In mitochondria of human cells, an increased level of AP sites results in quick loss of mtDNA, indicating that the DNA degradation is unlikely due to mitophagy, autophagy, or apoptosis [210, 388]. Using biochemical assays and mitochondrial extracts from human cells, a recent study demonstrated that TFAM accelerates the degradation of AP lesions containing DNA [209]. The study revealed that the stability of the AP site decreased tremendously upon binding to TFAM. The

half-life of AP lesions within TFAM-DNA complexes was 230- to 1,200-fold shorter relative to free AP-DNA, depending on the position of AP sites. TFAM-catalyzed AP-DNA destabilization occurred with mitochondrial LSP sequence or nonspecific DNA, and produced DNA SSBs and DNA-TFAM crosslinks. Upon treatment of AP-DNA with mitochondrial extracts of human cells, TFAM-DNA crosslink intermediates prior to the strand scission were also identified. The TFAM-catalyzed AP lesion destabilization was facilitated by lysine residues of TFAM to form Schiff base intermediates. Thus, data from the study suggested a novel role for TFAM in facilitating strand cleavage at AP sites, which may aid in the degradation of damaged mtDNA molecules [209].

7

SANITATION OF PREMUTAGENIC FREE NUCLEOTIDES

Free dNTPs, which are utilized as precursors for replication and repair, are under constant exposure to oxidation and other stresses [198]. Oxidation of the mitochondrial dNTP pool represents a major threat to mtDNA integrity [198] as damaged (predominantly, oxidized) dNTPs can substantially contribute to mismatch errors during DNA synthesis [204]. For example, 8-oxodeoxyguanosine triphosphate (8-oxodGTP) can be readily incorporated opposite a template A by pol γ, and the resulting 8-oxodG:dA base pairs are resistant to the intrinsic proofreading activity of pol γ, resulting in AT to CG transversions [415, 416]. *In vitro*, replication fidelity of pol γ was decreased when the content of 8-oxodGTP constituted as little as 0.06-0.6% of the total dGTP pool. Estimated 8-oxodGTP concentrations in the mitochondrial extracts from various rat tissues range from 1-10% of total dGTP that is about an equal level with deoxythymidine triphosphate (dTTP), with which it competes for incorporation opposite A in mtDNA [416]. To avoid misincorporation of potentially mutagenic dNTPs into mtDNA, different triphosphatases sanitize the dNTP pool in the mitochondrial compartment [206]. For instance, MTH1, the mammalian homolog of *E. coli* MutT protein, is a specialized

enzyme found in the cytosol as well as in mitochondria [417, 418], where it hydrolyzes 8-oxodGTP to 8-oxodeoxyguanosine monophosphate (8-oxodGMP). The latter nucleotide is not a substrate for pol γ and hence cannot be incorporated into the mtDNA [206, 217]. Moreover, MTH1 also hydrolyzes two major oxidation products of deoxyadenosine triphosphate (dATP), viz., 8-oxo-2′-deoxyadenosine triphosphate and 2-hydroxy-2′-deoxyadenosine triphosphate, to the monophosphates [418, 419]. Thus, MTH1 appears to play a significant general role in protecting mtDNA from mutagenic oxidized dNTPs. Consistent with this notion, an elevation in 8-oxoG level in mtDNA was noticed in dopaminergic neurons from *MTH1*-null mice upon administration with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and in *MTH1*-null mouse embryonic fibroblasts following H_2O_2 treatment, which were accompanied by mitochondrial dysfunction and cell death [420, 421]. MTH1 was also shown to protect cells from the cytotoxicity of sodium nitroprusside by preventing the accumulation of 8-oxoG specifically in mtDNA [422]. A study revealed an increased level of MTH1 in mitochondria of dopaminergic cells of patients with Parkinson's disease [423] indicating the significance of MTH1 in counteracting mitochondrial oxidative stress [205]. Interestingly, mitochondrial MTH1 is induced by ROS [424] suggesting that mitochondrial splice variants of nuclear repair genes can be specifically regulated in response to mitochondrial ROS levels.

As intracellular guanosine triphosphate (GTP) is much more abundant than dGTP, it is obvious that if dGTP is oxidized, GTP would also be oxidized [205]. Because 8-oxoGTP can cause transcription error [425], elimination of 8-oxoGTP may also be important in mitochondria. MTH1 protein harboring intrinsic 8-oxodGTPase activity also bears the potential to hydrolyze 8-oxoGTP to 8-oxoGMP, thereby preventing misincorporation of 8-oxoG into RNA [426].

Deoxyuridine triphosphate (dUTP) can be a substrate for DNA synthesis and can be incorporated against A [205]. Even though the incorporation of deoxyuridine monophosphate (dUMP) against A itself is not mutagenic, the A/U pairs are cleaved by UDG giving rise to AP sites or SSBs that are potentially mutagenic [205]. Indeed, an elevation of the dUTP/dTTP ratio has been known to result in DNA fragmentation and cell death [427]. The human *DUT* gene, which produces a dUTPase, eliminates dUTP arising from deamination of dTTP from the nucleotide pool, also encodes an alternative splice variant that is specifically translocated to mitochondria [428]. The mitochondrial protein is constitutively expressed, which is in contrast to the nuclear isoform that is cell cycle regulated [428]. Even though sanitation of the mitochondrial dNTP pool is not a DNA repair mechanism *per se*, it preserves mtDNA integrity through the reduction of mismatches in mtDNA, thereby reducing mutagenesis [204].

If altered dNTPs are incorporated into mtDNA, they must be removed via the BER pathway, which deals with modifications of single nucleotides already incorporated in DNA.

8
TRANSLESION SYNTHESIS

Translesion synthesis (TLS) refers to the capability of a polymerase to incorporate a nucleotide opposite a template lesion or adduct and extend the nucleotide past the lesion [429]. It is an error-prone process in which polymerases with low fidelity are involved [430]. This mechanism utilized by cells is an important strategy for alleviating DNA damage that cannot be repaired due to the chemical nature of the lesion [390].

The conversion of DNA lesions to mutations occurs frequently in the cell. For example, error-prone DSBR pathways may generate mutations; however, mutations may also be produced during DNA replication [10]. Bulky lesions block the advance of most replicative polymerases, as the strict substrate requirements of the active sites, and the existence of exonuclease proofreading domains prevent advance across the damaged region [431-433]. For replication to proceed, TLS occurs, which requires DNA polymerase selection and switching [434]. Specialized polymerases that can bypass lesions and copy defective templates have been detected in a variety of organisms from bacteria to humans. These translesion polymerases can permit replication to resume, although at the cost of reduced fidelity. These error-prone enzymes perform a significant role in the replication of the genome after damage,

as the majority of mutations produced by UV-light in the nucleus of yeast, for instance, rely on the presence of the error-prone polymerase, DNA polymerase zeta consisting of Rev3 and Rev7 subunits [435-437]. Even though these polymerases do not act in the repair of DNA, they are considered to take part in damage tolerance thus playing an important role in the cellular response to lesions [10]. Polymerases that have the capability to perform TLS in mitochondria are described ahead.

8.1 DNA Polymerase Gamma

Mitochondrial pol γ can bypass DNA lesions in mtDNA [206]. Initial *in vitro* studies with *X. laevis* pol γ revealed that AP sites inhibited 80% of strand synthesis; however, pol γ caused almost exclusive incorporation of dATP when TLS did occur [438]. Pol γ can also incorporate a dATP opposite an 8-oxodG [438] or introduce a random nucleotide opposite bulky DNA adducts derived from benzo[a]pyrene diol epoxide [439]. *In vitro* experiments with purified human pol γ suggest that this polymerase bypasses thymine dimers with drastically decreased efficiency when compared to undamaged templates [440]. On the other hand, purified pol γ can conduct error-prone synthesis across acrolein-derived DNA adducts at relatively high efficiency. Acrolein is a mutagenic aldehyde, which is produced endogenously by lipid peroxidation. It is also a common environmental toxin generated exogenously by the combustion of organic materials, including tobacco products. The error-prone bypass of these adducts by pol γ has been postulated to be an important source of damage-induced mutations in mammalian cells [441]. It should be noted that pol γ bypass through an AP site or 8-oxoG is still a matter of debate as different studies have produced conflicting results [438, 442]. A study utilized purified mitochondrial replisome, which consists of pol γ holoenzyme, mtSSB, and the replicative helicase Twinkle

to understand lesion bypass synthesis on oxidative damage-containing DNA templates [442]. Experiments were conducted at dNTP levels comparable to those prevailing either in cycling cells or in non-dividing cells. At "normal" dNTP concentrations that mimicked cycling cells, the mitochondrial replisome showed moderate stalling at 8-oxoG. At "low" dNTP concentrations mimicking resting cells, the stalling of the mtDNA replisome at 8-oxoG further exacerbated indicating that oxidative stress can cause mtDNA instability, especially in non-dividing cells. The study also revealed that an AP site poses a complete block for the proofreading proficient mtDNA replisome [442].

AP sites, which are normally repaired by the BER pathway, represent a block to both replication and transcription. However, when the number of AP sites overwhelms the BER pathway, mtDNA is targeted for degradation. A study utilized two UNG1 mutants to produce AP sites directly in the mtDNA of NIH3T3 (immortalized mouse embryonic fibroblast) cells *in vivo* at sites where T or C residues are normally present to understand the repair of these lesions in their natural context [390]. The study revealed that pol γ can perform TLS across AP sites in mitochondria of NIH3T3 cells, and preferentially inserts A opposite the AP site [390]. However, in that system, translesion bypass of AP sites occurred far less frequently than BER and mtDNA degradation, which are likely to be the major pathways for the processing of AP sites *in vivo* [390]. Most DNA polymerases of both pro- and eukaryotic origin most frequently incorporate an A opposite the AP site. This preferential incorporation of A has become known as the A-rule [443]. *In vitro*, *X. laevis* and human pol γ obey the A-rule [219, 438]. The study concluded that pol γ only inefficiently bypasses AP sites and that BER and mtDNA degradation are primarily responsible for the processing of the majority of AP sites in the mitochondrial genome *in vivo* [390].

8.2 PrimPol

Eukaryotic PrimPol is a recently discovered enzyme that has been detected to be active both in the nucleus and mitochondria. This enzyme, as its name suggests, has DNA-dependent DNA primase activity as well as DNA-dependent DNA polymerase activity [444]. The main function of PrimPol is to rescue stalled replication forks at bulky lesions either by bypassing the damage via TLS or adding primers downstream of the damage and reinitiating replication. Loss of PrimPol results in impaired mtDNA replication, slower replication fork progression, drastically decreased mtDNA levels, and increased sensitivity to DNA damaging agents [444-446]. Moreover, knockdown of PrimPol in mouse and human cells causes a reduction in overall mtDNA levels and inhibits cell's capabilities to recover after chemical depletion of mtDNA [444]. PrimPol has been shown to functionally interact with different proteins in the mitochondrial compartment. These include mtSSB and Twinkle, which seem to play roles in regulating PrimPol's cellular activities [81, 442, 447]. The presence of PrimPol in both the nucleus and mitochondria indicates that it plays similar roles in maintaining DNA integrity in both compartments [444, 445].

PrimPol's primase activity is unique for eukaryotes as it can utilize either nucleoside triphosphates (NTPs) or dNTPs for primer synthesis on ssDNA templates [444]. Although PrimPol can generate both DNA and RNA primers, it has a strong preference for utilizing dNTPs rather than NTPs to produce primers [81] [444]. Emerging data imply that PrimPol's main role is likely to be in repriming replication restart following a fork-stalling lesion or DNA structure [448, 449]. PrimPol appears to play a similar role within the mitochondrion as it performs in the nucleus, where it rescues stalled replication fork via repriming events ahead of bulky DNA adducts [429]. A recent study has revealed that PrimPol can reinitiate stalled mtDNA replication *in vivo* and *in*

vitro and can prime mtDNA replication from non-conventional origins. PrimPol is specifically required for replication initiation following DNA damage. For PrimPol to rescue blocked replication forks in mitochondria, pol γ should be able to utilize the primers generated by PrimPol. Indeed, this was revealed to be the case, as pol γ extended DNA primers constructed by PrimPol on ssDNA template [450].

In addition to its ability to produce primers, PrimPol is also a template-dependent DNA polymerase with the capability to bypass some lesions. For instance, it can perform TLS bypass of 8-oxoG lesions but is unable to bypass AP sites or thymidine glycol lesions unless supplemented with manganese [444, 445]. Moreover, PrimPol can bypass damage caused by UV exposure and, unlike known mammalian polymerases, PrimPol has the ability to bypass distorting 6-4PP lesions that induce DNA bending [445]. Furthermore, it has been reported that PrimPol can scrunch the template, realigning the priming strand so as to bypass intolerable DNA lesions, which appears to be the more likely mode of bypass given the protein's small active-site cleft [451, 452]. Therefore, it is plausible that PrimPol may also play a role in the TLS bypass of these types of damage within the mitochondrial genome and may be the main source of mitochondrial lesion bypass in mammals.

PrimPol is an extremely unprocessive polymerase, catalyzing the incorporation of only 1-4 nucleotides before dissociating from DNA, and is also highly error-prone, particularly susceptible to produce insertion/deletion errors [446, 447, 451]. Whilst TLS performed by PrimPol is highly mutagenic [447], repriming events by PrimPol downstream of an AP site lead to error-free resumption of replication, resulting in an overall anti-mutagenic effect [453]. These unique attributes imply a significant role of PrimPol in the maintenance of nDNA and probably mtDNA as well.

Even though PrimPol is proposed to have a nuclear role in TLS, to date there is no evidence for this role in mitochondria. In fact, a recent *in vitro* study revealed that the addition of PrimPol to the mitochondrial replisome does not enhance replication past an 8-oxoG or an AP site [442]. However, the study was conducted without any potential co-factors for PrimPol, which may be necessary for any mitochondrial TLS.

8.3 DNA Polymerase Zeta

DNA polymerase zeta (pol ζ) is an error-prone TLS polymerase comprising of two subunits: the catalytic subunit Rev3 and the structural subunit Rev7 [454]. Rev3 has been revealed to be important in the bypass of UV- and chemically-induced DNA damage; however, this bypass typically leads to mutations [455-457]. Following exposure to UV-light, mutations, both deletions and base substitutions, have been revealed to accumulate in human skin, indicative of error-prone bypass [458-460]. The regulation of *Rev3* expression seems to be important for genome stability. While deletion of *Rev3* is embryonic lethal in mice and leads to chromosomal instability in human and mouse cells [461-464], overexpression of *Rev3* results in increased spontaneous mutations, and is associated with different types of cancer [465-467]. Rev3 has been long known to act as a nuclear TLS protein, and it has recently been suggested that Rev3 also localizes to mitochondria in humans [468] and yeast [469] to participate in TLS on mtDNA. Even though Rev7 has been suggested to localize to yeast mitochondria [469], there is no such evidence indicating the presence of Rev7 in mitochondria in humans.

In 2015, the interplay between *Rev3* expression and mitochondrial function was demonstrated [468]. Rev3 associated with pol γ and mtDNA, and protected the mitochondrial genome from DNA damage. Deletion of *Rev3* in MEF cells revealed reduced

levels of COX II mRNA and protein, and decreased complex IV activity. MtDNA copy numbers were also reduced in Rev3$^{-/-}$ cells. Moreover, the Rev3$^{-/-}$ cells showed signs of mitochondrial distress with increased glucose consumption rates, lower mitochondrial membrane potential, and decreased ROS levels, all probably due to decreased complex IV activity. On the contrary, *Rev3* expression was increased in cells administered with inhibitors of OXPHOS and ρ^0 cells, indicating that higher Rev3 levels can help compensate for decreased OXPHOS [468].

The researchers endeavored to reveal that Rev3 directly acts on mtDNA to help protect it from damage induced by UV-exposure [468]. In cells expressing Rev3 devoid of the MTS, mtDNA had increased levels of lesions after UV-exposure. Chromatin immunoprecipitation assay revealed that Rev3 was bound to both the D-loop and COX II regions of mtDNA. As these were the only two regions presented in the study, a comprehensive picture of Rev3 binding sites on mtDNA is still lacking [468].

The study also revealed increased expression of *Rev3* in human primary breast tumors and breast cancer cell lines [468]. Inactivation of *Rev3* decreased cell migration and invasion, and localization of Rev3 in mitochondria increased survival and the invasive potential of cancer cells. Thus, the study was able to demonstrate that mitochondrial Rev3 is associated with the tumorigenic potential of cells [468].

8.4 DNA Polymerase Theta

DNA polymerase theta (pol θ) was recently identified in mitochondria of human cells [470]. In the nucleus, pol θ has been implicated in BER, DSBR, NHEJ, and maintenance of DNA replication timing [471-476]. It is a proofreading-deficient and error-prone polymerase that can perform translesion DNA polymerization [477]. Compared to pol γ, pol θ has low fidelity

and has only moderate processivity [477, 478]. Pol θ is able to perform TLS by incorporating bases opposite an AP site or a thymine glycol residue in the template strand and elongating an unpaired primer base opposite these lesions [479]. However, pol θ is unable to incorporate bases opposite a CPD or a 6-4PP [480]. Although it does not contain an MTS, pol θ is localized to mitochondria following oxidative damage [470], implying that the enzyme is recruited to the organelle when the damage of this type is inflicted, where it potentially facilitates TLS [331]. Intriguingly, loss of pol θ results in a decrease in cellular oxygen consumption and mitochondrial membrane potential, suggestive of decreased OXPHOS [470]. In these pol θ expression lacking cells, the rate of the point mutation in mtDNA is substantially decreased following oxidative treatment [470], indicating that pol θ participates in error-prone DNA synthesis that may facilitate replication in mitochondria [331].

It is now becoming clear that similar to the nucleus, mitochondria also utilize a wide range of tolerance mechanisms to overcome DNA damage, which may occur spontaneously or arise due to different environmental factors. The existence of multiple TLS polymerases within the mitochondrial compartment implies that these organelles also utilize such specialized polymerases to overcome DNA lesions [81].

ABBREVIATIONS

ρ^+	rho positive
ρ^0	rho zero
3-MeC	3-methylcytosine
6-4PP	pyrimidine-pyrimidone (6-4) photoproduct
8-OHG	8-hydroxyguanine
8-oxodG	8-oxodeoxyguanosine
8-oxodGMP	8-oxodeoxyguanosine monophosphate
8-oxodGTP	8-oxodeoxyguanosine triphosphate
8-oxoG	8-oxyguanine
A	adenine
AAG	alkyladenine DNA glycosylase
Abf2	ARS-binding factor 2
A-NHEJ	alternative non-homologous end joining
ANT	adenine nucleotide translocator
AP	apurinic/apyrimidinic/abasic
APE1	apurinic/apyrimidinic endonuclease 1
ARS	autonomously replicating sequence
AT	adenine-thymine
ATAD3	ATPase family AAA-domain-containing protein 3
ATP	adenosine triphosphate
ATP6	ATP synthase subunit 6
ATP8	ATP synthase subunit 8
ATP9	ATP synthase subunit 9
BER	base excision repair

bp	base pair
C	cytosine
CM	cristae membrane
C-NHEJ	classical non-homologous end joining
COB	apocytochrome *b*
COX1	cytochrome *c* oxidase subunit 1
COX2	cytochrome *c* oxidase subunit 2
COX3	cytochrome *c* oxidase subunit 3
CPD	cyclobutane pyrimidine dimer
CSA	Cockayne syndrome Group A
CSB	Cockayne syndrome Group B
dATP	deoxyadenosine triphosphate
dGTP	deoxyguanosine triphosphate
D-loop	displacement loop
DNA2	DNA replication ATP dependent helicase/nuclease 2
DNA-PK	DNA-dependent protein kinase
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
dNTP	deoxyribonucleoside triphosphate
DR	direct repair/direct reversal
dRP	deoxyribose phosphate
DSB	double-strand break
DSBR	double-strand break repair
dsDNA	double-stranded DNA
dTTP	deoxythymidine triphosphate
dUMP	deoxyuridine monophosphate
dUTP	deoxyuridine triphosphate
ETC	electron transport chain
EXOG	exo/endonuclease G
FapyG	2,6-diamino-4-hydroxy-5-formamidopyrimidine
FEN1	flap endonuclease 1
G	guanine
GC	guanine-cytosine
GTP	guanosine triphosphate

HMG	high mobility group
HNSCC	head and neck squamous cell carcinoma
HO$^{\cdot}$	hydroxyl radical
H_2O_2	hydrogen peroxide
HR	homologous recombination
HSP1	heavy strand promoter 1
HSP2	heavy strand promoter 2
HSP60	heat-shock protein 60
H-strand	heavy strand
IDL	insertion/deletion loop
IR	ionizing radiation
kb	kilobase pair
LIG3	DNA ligase III
LP-BER	long-patch base excision repair
LSP	light strand promoter
L-strand	light strand
MGME1	mitochondrial genome maintenance exonuclease 1
MGMT	O^6-methylguanine DNA methyltransferase
mito*Pst*I	mitochondrial-targeted restriction endonuclease *Pst*I
MLH	MutL homolog
MMEJ	microhomology-mediated end joining
MMR	mismatch repair
MNF1	mitochondrial nucleoid factor 1
MPG	methylpurine DNA glycosylase
MRN	Mre11, Rad50, Nbs1
MRX	Mre11, Rad50, Xrs2
MSH	MutS homolog
mtDNA	mitochondrial DNA
mt-nucleoid	mitochondrial nucleoid
mt-ribosome	mitochondrial ribosome
MTS	mitochondrial targeting sequence
mtSSB	mitochondrial single-stranded DNA-binding protein
MUTYH	MutY homolog DNA glycosylase

Nbs1	Nibrin
NCR	noncoding region
nDNA	nuclear DNA
NEIL1	endonuclease VIII-like glycosylase 1
NEIL2	endonuclease VIII-like glycosylase 2
NEIL3	endonuclease VIII-like glycosylase 3
NER	nucleotide excision repair
NHEJ	non-homologous end joining
NTH1	endonuclease III-like protein 1
NTP	nucleoside triphosphate
$^{\bullet}O_2^-$	superoxide anion radical
***O*^{6}-BudG**	*O*^{6}-butyl-2′-deoxyguanosine
OGG1	8-oxoguanine DNA glycosylase
OH	hydroxyl
O_H	heavy strand origin of replication
O_L	light strand origin of replication
OXPHOS	oxidative phosphorylation
PARP1	poly-ADP-ribose polymerase
PNKP	polynucleotide kinase/phosphatase
pol β	DNA polymerase beta
pol γ	DNA polymerase gamma
pol ζ	DNA polymerase zeta
pol θ	DNA polymerase theta
POLRMT	mitochondrial RNA polymerase
ROS	reactive oxygen species
rRNA	ribosomal RNA
siRNA	small interfering RNA
SP-BER	short-patch base excision repair
SSB	single-strand break
ssDNA	single-stranded DNA
T	thymine
TC-NER	transcription-coupled nucleotide excision repair
TFAM	transcription factor A, mitochondrial

TFB2M	transcription factor B2, mitochondrial
TLS	translesion synthesis
tRNA	transfer RNA
UDG	uracil DNA glycosylase
UHA	unsaturated hydroxyaldehyde
UNG1	uracil-N-glycosylase 1
UNG2	uracil-N-glycosylase 2
UV	ultraviolet
XPA	Xeroderma Pigmentosum Group A
XPD	Xeroderma Pigmentosum Group D
XPG	Xeroderma Pigmentosum Group G
YB-1	Y-box binding protein 1

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